OPTICAL AND HYDRODYNAMIC PROPERTIES OF POLYRIBONUCLEOTIDES

by

G. C. Chen
B.S., National Taiwan University, 1966

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOCHEMISTRY

in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA

Approved: Jen Tsi Yang
Janakiraman Ramachandran
Martin D. Sheetz
Committee in Charge

Deposited in the Library, San Francisco Medical Center:

January 4, 1973 Jeanette G. Frazee
Date Librarian

Degree Conferred: September 20, 1972

DISSERTATION ABSTRACT

OPTICAL AND HYDRODYNAMIC PROPERTIES OF POLYRIBONUCLEOTIDES

G. C. Chen

DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS

UNIVERSITY OF CALIFORNIA

SAN FRANCISCO

The optical and hydrodynamic properties of four polyribonucleotides, poly(A), poly(G), poly(C), and poly(U), were studied. Changes in size, shape, and conformation of these polymers in solution in response to changes in pH or temperature were determined by ultraviolet absorption, circular dichroism (CD), optical rotatory dispersion (ORD), viscosity, and sedimentation. The molecular weight of these polynucleotides was estimated from the β-function, assuming a prolate ellipsoid of revolution, to ascertain whether conformational changes under various conditions are accompanied by changes in the size of the molecules. The molecular weight based on the β-function agreed with that determined by sedimentation equilibrium.

Poly(A) in acidic solution exhibits a bell-shaped curve when either the intrinsic viscosity or the extrema of ORD and CD are plotted against the pH of the solution. The first cooperative transition occurs at pH 5.7 to 5.9 (in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid). Apparently, the single-stranded poly(A) at neutral pH is dimerized to form an extended double-stranded helix when the adenine bases are half-protonated. Further protonation of the bases induces a second transition at pH 5.0 to 5.2, in which poly(A) remains double-stranded

but the polynucleotide chain becomes more compact and more symmetric, probably because of additional "neutralization" of the opposite charges on adenine and phosphate groups.

Poly(G) at neutral pH is highly aggregated and shows an extremely high thermal stability. In alkaline solution above pH 11, it undergoes a time-dependent disaggregation. The rate of dissociation increases at higher pH and temperature. Deprotonation of the guanine bases increases electrostatic repulsion among the nucleotide residues. Thus, poly(G) in alkaline solution, unlike the other polynucleotides studied, has little, if any, base stacking. In acidic solution poly(G) also undergoes a reversible transition near pH 3 in 0.01 M sodium citrate-HCl buffer, suggesting some changes in its conformation. But in contrast to poly(G) in alkaline solution there is no evidence of disaggregation.

Poly(C) undergoes a transition upon half protonation of the cytosine bases. The midpoint of the transition occurs at pH 5.7-5.8 in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid. With this transition the molecular weight of poly(C) does not change with pH, but the intrinsic viscosity drops markedly. These results suggest that poly(C) forms a hairpin structure upon half protonation, although X-ray studies by other investigators of poly(C) fibers drawn from concentrated acidic solution indicated a parallel, double-stranded helix. Our finding is not necessarily inconsistent with the X-ray results, however, since two single-stranded hairpin molecules could conceivably be stretched into a double-stranded complex.

Poly(U) at room temperature is known to lack any secondary structure, but the appearance of multiple Cotton effects suggests some degree of base stacking. Lowering the temperature of the solution progressively toward 0°C induces a thermal transition to a more ordered

structure. The CD spectrum at low temperature suggests the formation of base pairs, but the molecular weight of poly(U) remains unchanged between zero and 25°C. Our results thus support the current hypothesis of a hairpin structure for poly(U) at low temperature.

Janakiraman Ramachandran

ACKNOWLEDGMENTS

I am greatly indebted to Professor J.T. Yang for his tireless interest, and constant encouragement. I wish to thank my thesis commitee members, Professors J. Ramachandran and M.D. Shetlar, for their valuable critique. Also, I wish to thank Professor L.M. Peller for his consultations. I am grateful to Drs. K.H. Chau and E. Daniel for their invaluable help with the scanner on the model-E ultracentrifuge, and to Miss C. Ue for her technical assistance. I also wish to thank Dr. K.H. Chau and Miss B.D. Wells for reading parts of the manuscript. I am deeply indebted to Dr. J.M. Quivey, my four-year roommate, for translating my Oriental thoughts to Occidental expressions. Finally, I wish to thank a wonderful couple, Mr. and Mrs. F.L. Smith, (my American host family), for their care and hospitality throughout my studies.

ABBREVIATION

poly(A)	polyriboadenylic acid
poly(C)	polyribocytidylic acid
poly(G)	polyriboguanylic acid
poly(U)	polyribouridylic acid
CD	circular dichroism
ORD	optical rotatory dispersion

The abbreviations for polyribonucleotides follow the Revised Tentative Rules of IUPAC-IUB Commission on Biochemical Nomenclature, 1971.

TABLE OF CONTENTS

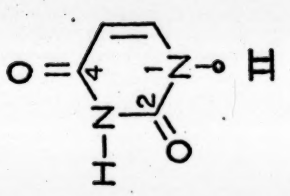
I. INTRODUCTION

Synthetic polyribonucleotides serve as simplified models for ribonucleic acid (RNA) structures. Much of what is known about the conformation of nucleic acids in solution is the result of extensive investigation on the chemical and physical properties of polynucleotides and their complexes. Polyribonucleotides can adopt different conformations under various experimental conditions such as change in temperature, pH, and solvent, and a great deal of attention has been focused on their structural changes. The reactions leading to changes in polyribonucleotide structures may occur according to a cooperative or a non-cooperative process (Felsenfeld & Miles, 1967; Michelson et al., 1967). The study of conformations and structural transitions in polyribonucleotides is important for a better understanding of the properties of the more complicated natural nucleic acids which contain a multiplicity of bases.

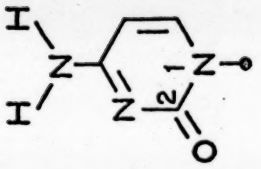
In this work, we study the optical and hydrodynamic properties of four polyribonucleotides: poly(A), poly(G), poly(C) and poly(U). The primary structure of polyribonucleotides is a linear homopolymer of nucleotides that contain purine or pyrimidine bases. The D-ribose is attached to the N(9) of the purine or the N(1) of the pyrimidines, and the phosphate group is attached to the 3' - or 5' - position of the pentose ring. The chemical structures and the numerical designation of the bases constituting the polyribonucleotides used in this study are shown in Figure 1.

The purine and pyrimidine bases have strong absorption bands near 260 nm and are optically inactive. These chromophores, however, become optically active upon interaction with two sources of asymmetry in the polyribonucleotide molecules: first, the pentose rings containing

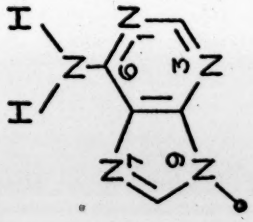
Figure 1: Chemical structure of the bases of polyribonucleotides. I, adenine; II, uracil; III, guanine; IV, cytosine.



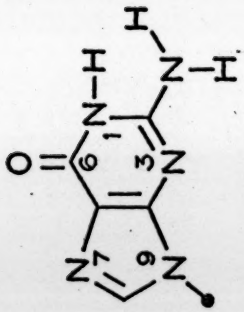
II



IV



I



III

asymmetric carbons and second, the overall dissymmetric helical conformation of the polymer. Optical properties (ORD, CD, and hypochromism) thus, reflect the molecular dissymmetry in solution, and, more importantly, the conformational changes due to any effect upon its environment. On the other hand, hydrodynamic properties (sedimentation and viscosity) give information about the size and shape of the polymer.

A. Polyriboadenylic Acid

Fresco and Doty (1957) first discovered that poly(A) could exist in two forms depending on the pH of the solution. They studied the molecular-weight dependence of the sedimentation coefficient and intrinsic viscosity and concluded that poly(A) at room temperature existed as a randomly coiled single chain in neutral solution and a rigid helical form in acidic solution. X-ray diffraction studies of poly(A) fibers prepared from acidic solution indicated a double-stranded parallel helix (Rich et al., 1961). Light scattering studies of poly(A) in neutral solution also suggested a coil model (Steiner & Beers, 1957). However, at neutral pH, poly(A) was found to be hypochromic to its monomer as first reported by Warner (1957) indicating the presence of some ordered structure (Steiner & Beers, 1961).

The studies of ORD and CD of poly(A) at neutral pH and room temperature indicated that it had an ordered dissymmetrical structure. Sarkar and Yang (1965a) and Lamborg et al. (1965) first reported the ORD of poly(A) above 200 nm and showed that at neutral pH, poly(A) possessed multiple Cotton effects (see also Holcomb & Tinoco, 1965; Michelson et al., 1966; Ts'o et al., 1966; Hashizume & Imahori, 1967; Adler et al., 1969). Below 200 nm, Ts'o et al. (1966) found another

large peak near 195 nm. Also, Brahms (1964) found that between 230 and 300 nm, poly(A) at pH 7.4 had an intense CD curve composed of a positive band at 264 nm and a negative one at 247 nm. Recently, the CD measurements were extended to 190 nm and showed another pair of bands below 230 nm (Hashizume & Imahori, 1967; Wolfe et al., 1969; see also Adler et al., 1969; Bush & Scheraga, 1969). Furthermore, the polymer of N(6)-hydroxyethyladenine which could not form hydrogen-bonded double-stranded helices, exhibited similar CD to that of adenylyl (3' - 5') adenosine, ApA (at neutral and acidic pH) and poly(A) (at neutral pH) (Van Holde et al., 1965). Further studies of optical properties of ApA (Holcomb & Tinoco, 1965; Warshaw et al., 1965; Van Holde et al., 1965) and of a series of adenylate oligomers (Cantor & Tinoco, 1965; Brahms et al., 1966; Cantor et al., 1966; Leng & Felsenfeld, 1966; Michelson et al., 1966) indicated that the structure of poly(A) at neutral pH was an extension of that of the dimer, ApA which was stabilized by the pair-wise stacking interaction between adjacent bases. Thus, poly(A) at neutral pH and room temperature was proposed to be a flexible single-stranded helix, stabilized by stacking of the bases which are perpendicular to the helical axis (Tinoco, 1964). Small-angle X-ray scattering studies also showed poly(A) at neutral pH to be a rod-like structure with the base plane perpendicular to the rod axis (Luzzati et al., 1964; Witz & Luzzati, 1965).

As the pH of poly(A) solution was lowered, with the consequent protonation of the adenine residue [-C(2)-N(1)=C(6)- + $H^+ \rightleftharpoons$ -C(2)-N(1)$^+$H=C(6)-] (Dekker, 1960; Ts'o et al., 1966), a double-stranded base-stacked structure formed (Fresco & Klemperer, 1959; Holcomb & Tinoco, 1965). Fresco (1959) had assumed that this structure was similar to, if not identical with, that found in fibers drawn from

acidic solution (a double-stranded parallel helix with base plane slightly tilted against the helical axis and stabilized by four interchain hydrogen bonds and two salt linkages) (Rich et al., 1961). Small-angle X-ray scattering studies confirmed these findings (Luzzati et al., 1964; Witz & Luzzati, 1965).

This acid-induced conformational change of poly(A) appeared to be a cooperative process as indicated by the sharp spectrophotometric and potentiometric titration curves (Beers & Steiner, 1957; Fresco & Klemperer, 1959; Steiner & Beers, 1959; Rawitscher et al., 1963; Holcomb & Timasheff, 1968; Adler et al., 1969). Lowering the pH of the poly(A) solution caused an abrupt decrease in the absorbance at λ_{max} as well as blue shift of the spectrum (Beers & Steiner, 1957; Fresco & Klemperer, 1959; Helmkamp & Ts'o, 1962). Brahms (1963, 1964) found that the CD of poly(A) in acidic solution showed a blue-shift and the first positive band near 260 nm was very much intensified when compared to that at neutral pH (see also Brahms & Mommaerts, 1964; Wolfe et al., 1969).

The ORD spectra of poly(A) in acidic solution as reported by various laboratories under different experimental conditions (Holcomb & Tinoco, 1965; Sarkar & Yang, 1965a; Ts'o et al., 1966; Green & Mahler, 1970) at pH values varying from 5.5 to 4.6 did not agree well on the Cotton effect between 280-290 nm. Recently, Adler et al. (1969) further studied the pH effect on the ORD of poly(A) and reported that it could exist in two different acidic forms. As the pH was lowered to about the pK_a value (5.87 in 0.1 M NaCl), there was a transition to an acidic form, designated as B form, with larger magnitude and red-shift of the peak compared to the neutral form. As the pH was further lowered, there was a gradual transition of the peak to a still more acidic form, A, which was blue shifted compared to the neutral form and was of even

larger magnitude than that for B form. Both acidic forms (A & B) had the same UV absorption spectrum which differed from that of the neutral form and their ORD curves were more similar to each other than to that of the neutral form. Therefore, Adler et al. (1969) concluded that B form was a double-stranded half-protonated structure, and A form that of complete protonation. Furthermore, at intermediate pH values, an equilibrium existed between the two forms which were reflected in the two peaks of various proportion (Adler et al., 1969). Thus, poly(A) was believed to change from a single-stranded to a double-stranded helix upon protonation of the adenine bases. Some increase in molecular weight was always observed and its magnitude increased sharply with increasing concentration of poly(A) (Steiner & Beers, 1961). Under appropriate conditions, an increase of tenfold or more in molecular weight had been observed (Fresco & Doty, 1957).

B. Polyriboguanylic Acid

At neutral pH, poly(G) has a secondary structure of high thermal stability, as indicated by the marked hypochromism relative to the monomer, guanosine monophosphate (GMP) (Fresco & Massoulie', 1963; Pochon & Michelson, 1965). Thermal denaturation of poly(G) at pH 7 (0.002 M Na^+ ion) was not found to be complete even at 100°C, as demonstrated by the hyperchromic effect (Fresco & Massoulie', 1963); however, in the absence of added salt at pH 6, the melting curve was complete above 90°C (Pochon & Michelson, 1965). As the pH was raised above 7, the thermal denaturation occurred at lower temperature, but increasing the ionic strength could counteract, in part, the effect of elevated pH (Fresco & Massoulie', 1963). However, the thermal denaturation appeared to be a non-cooperative process in low ionic strength

(Pochon & Michelson, 1965; Green & Mahler, 1970).

Alkaline spectrophotometric titration of poly(G) [-N(1)H-C(6)=O + $OH^- \rightleftarrows$ -N(1)=C(6)$^-$-O + H_2O] (Tsuboi et al., 1962; Shapiro, 1968) was found to be abrupt from a highly ordered to a disordered conformation (Fresco & Massoulie', 1963; Pochon & Michelson, 1965). The pH at the midpoint of alkaline titration, designated as alkaline pK_a, was dependent on the ionic strength used (Pochon & Michelson, 1965): 11.86 in 0.01 M, 11.43 in 0.15 M and 11.32 in 0.25 M NaCl. This shift in pK_a value was presumably a consequence of the reduced electrostatic repulsion of ionized phosphate groups in salt concentration (Michelson, 1963). In contrast, the alkaline pK_a value of the monomer, GMP was only 9.4 in 0.2 M NaCl (Fresco & Massoulie', 1963). Poly(G) at pH above the alkaline pK_a displayed only a slight hypochromism relative to GMP unlike that at neutral pH (Fresco & Massoulie', 1963).

Pochon & Michelson (1965) found that acidic spectrophotometric titration of poly(G) [=N(7)- + $H^+ \rightleftarrows$ =N(7)$^+$H] (Dekker, 1960; Tsuboi et al., 1962; Shapiro, 1968) was non-cooperative and indicated no loss of secondary structure. They therefore concluded that the removal of a proton on N(1), but not the addition of a proton to N(7), led to an abrupt collapse of secondary structure. The acidic pK_a value of poly(G) determined spectrophotometrically was highly dependent on the ionic strength used (Thiele & Guschlbauer, 1971) just as in the case of alkaline titration; namely, the higher the ionic strength the lower the pK_a value.

Ulbricht et al. (1966) found that at neutral pH, poly(G) exhibited an ORD spectrum of multiple Cotton effects. The rotation changed little as the pH was reduced; and the positions of the extrema were red-shifted at pH 2. In contrast, at pH 12.2 the ORD spectrum displayed only a

single, negative Cotton effect similar to that of the purine β-mononucleotides, thus indicating a complete loss of the secondary structure. Arya (1968) and Green & Mahler (1970) reported the same finding for poly(G) at neutral pH, except that the ORD extrema were located several nanometers bathochromic to those reported by Ulbricht et al. (1966). The corresponding CD spectrum of poly(G) at pH 7 also showed multiple Cotton effects between 310 and 210 nm (Arya, 1968; see also Green & Mahler, 1970).

Wolfe et al. (1969) have extended the CD measurements of poly(G) at both neutral and acidic pHs to 185 nm. Their results at pH 7 and pH 3.4 (in 0.1 M phosphate buffer) above 220 nm were essentially the same as those previously reported in neutral pH (Arya, 1968; Green & Mahler, 1970). The CD at pH 7 was salt-independent. However, at pH 3.3 with no added salt, the shape of the spectrum was inverted, viz., the band on the longer wavelength side, centered at 285 nm, was negative in sign, followed by a positive band at 255 nm (see also Thiele & Guschlbauer, 1971). A similar change in ORD of poly(G) was observed between pH 6.8 and 2.2 in 0.01 M sodium citrate-HCl buffer (Cohen et al., 1969). Multiple-stranded association was suggested for the inverted type of spectrum (Brahms & Brahms, 1970).

C. Polyribocytidylic Acid

Fasman et al. (1964) found that the ORD of poly(C) at pH 7 had a single Cotton effect between 350 and 255 nm, which remained unchanged in the presence of formaldehyde (a reagent which blocks the amino group of cytidine). Thus, poly(C) at neutral pH existed as a single-stranded helix, in which base stacking rather than hydrogen bonding was responsible for maintaining the helical structure. Sarkar and

Yang (1965b) extended the ORD measurements to lower wavelength and found two shoulders between 250 and 220 nm, indicating that poly(C) at neutral pH also had multiple Cotton effects (see also Ts'o et al., 1966; Adler et al., 1967; Green & Mahler, 1970; Sarocchi et al., 1970; Gulik et al., 1970). The ORD remained essentially the same when the pH was raised from neutral to pH 12.2 (Ulbricht et al., 1966). The CD of poly(C) at neutral pH was found to be nonconservative (Brahms et al., 1967; Adler et al., 1968; Wolfe et al., 1969; Gulik et al., 1970; Sarocchi et al., 1970; Thiele & Guschlbauer, 1971). In slightly alkaline solution from pH 8 to 10, the CD of poly(C) was the same as that at neutral pH (Adler et al., 1968; Green & Mahler, 1970).

Poly(C) at neutral pH showed a gradual hyperchromic effect as well as a gradual decrease in the Cotton effects with increasing temperature (Ts'o et al., 1962, 1966; Fasman et al., 1964; Sarkar & Yang, 1965b; Gulik et al., 1970), indicating a non-cooperative melting process which was reversible upon cooling (Adler et al., 1967; Gulik et al., 1970). Furthermore, the melting process was concentration independent and thusly ruled out any intermolecular complex formation (Adler et al., 1967). According to Michelson et al. (1967) poly(C) at neutral pH could best be considered as a random coil, with continuously fluctuating regions of helices arising from base-base interactions. Small-angle X-ray scattering studies of poly(C) at neutral pH indicated that the molecule was rod-like, with the planar bases at least partially stacked and somewhat tilted with respect to the axis of the rod (Gulik et al., 1970).

In acidic solution, the absorption spectrum of poly(C) was red-shifted and showed a hyperchromic effect relative to that in neutral solution (Helmkamp & Ts'o, 1962; Guschlbauer, 1967). Studies

of thermal stability at various pH and ionic strength led Akinrimisi et al. (1963) to suggest that poly(C) in acidic solution formed a double-stranded helix with a pair of hydrogen bonds between the amino [-C(4)-NH_2] and keto [-C(2)=O] groups of the two cytosine residues and a third hydrogen bond with a shared proton in between. X-ray diffraction studies of poly(C) fibers drawn from acidic solution reached the same conclusion and, furthermore, the double-stranded helix was of parallel type (Langridge & Rich, 1963).

The ORD of poly(C) in acidic solution was significantly red-shifted and was accompanied by a downward displacement of the main Cotton effect between 350 and 260 nm (Fasman et al., 1964; Guschlbauer, 1967). This was quite different from the protonation of poly(A) which resulted in a slight blue-shift of the ORD and an increase in the magnitude of the Cotton effects (see Section IA). Sarkar and Yang (1965a) suggested that the change in ORD of poly(C) from neutral to acidic solution could be due to both protonation and change in secondary structure. The ORD below 260 nm of poly(C) in acidic solution was complicated showing three to four shoulders (Ulbricht et al., 1966; Ts'o et al., 1966; Adler et al., 1967; Sarocchi et al., 1970).

Brahms et al. (1967) found that the CD of poly(C) in acidic solution was conservative with a pair of bands opposite in sign (see also Sarocchi et al., 1970; Thiele & Guschlbauer, 1971). The CD spectrum was red-shifted as was the entire absorption spectrum. Wolfe et al. (1969) extended the CD measurements to 185 nm and found that the large symmetrical Cotton effect centered at 210 nm at neutral pH disappeared. The studies of a series of oligocytidylates at acidic pH showed that the appearance of the 265-nm negative band started with the heptamer, which was thought to be the lower

limit for the formation of the double-stranded acidic structure (Brahms et al., 1967).

Potentiometric titration of poly(C) [-C-N(3)=C- + $H^+ \rightleftarrows$ -C-N(3)$^+$H=C-] (Dekker, 1960) showed two abrupt steps that are characteristic of a cooperative transition (Hartman & Rich, 1965). In connection with thermal denaturation studies, Hartman and Rich (1965) concluded that the addition of the first proton per base pair stabilized the acidic double-stranded helix, but the addition of the second one destabilized it (see also Guschlbauer, 1967).

Hydrodynamic studies also revealed a different conformation of poly(C) in neutral and acidic solution. The intrinsic viscosity of poly(C) decreased (Akinrimisi et al., 1963) and the sedimentation coefficients increased (Akinrimisi et al., 1963; Hartman & Rich, 1965) when the pH was lowered. These results were in accord with the observation of a reduction in radius of gyration of poly(C) as it changed from neutral to acidic form (Steiner & Beers, 1957).

D. Polyribouridylic Acid

Lipsett (1960) first described the disorder-order transition of poly(U) by following the UV absorbance towards 0°C. This transition was markedly dependent on the salt concentration as well as the nature of the ions. Divalent cations were more effective on these changes than monovalent ones; for example, 10^{-3} M $MgCl_2$ produced approximately the same amount of hypochromicity (about 25%) as 1 M NaCl (Lipsett, 1960). The effect of monovalent cations on the melting temperature, T_m, of poly(U) was $Cs^+ > K^+ > Na^+ > Li^+$, whereas for other polynucleotides the order was just the reverse (Brown, 1966; Leng & Michelson, 1968).

The UV spectrum of poly(U) at room temperature showed little hypochromicity with respect to the monomer (Warner, 1957). Richards et al. (1963) found that the UV absorbance of poly(U) had only a small temperature dependence above 15°C. In connection with hydrodynamic measurements, they concluded that this polymer possessed no base stacking at room temperature (see also Simpkins & Richards, 1967a). This conclusion was challenged by Michelson and Monny (1966), who claimed that the small hypochromism with respect to temperature might arise from base interactions. Even at temperatures above 15°C, increasing the salt concentration above 0.5 M NaCl did cause a small decrease in absorptivity of poly(U) at 260 nm (Simpkins & Richards, 1967b; Inners & Felsenfeld, 1970), suggesting that base-stacking, if any, was favored only in high salt concentration. In spite of the controversy raised by Michelson and Monny (1966) about the conformation of poly(U), most physical studies seemed to suggest that at room temperature and neutral pH, poly(U) was essentially a single-stranded random coil devoid of significant secondary structure (Richards et al., 1963; Simpkins & Richards, 1967a, b; Inners & Felsenfeld, 1970).

The formation of an ordered structure of poly(U) could also be followed with its ORD and CD. The variation of the Cotton effects with temperature depended on the ionic strength and the nature of salt used. The ORD of poly(U) exhibited multiple Cotton effects, and the rotation was essentially the same between 20 and 1°C in 0.05 M Na^+ salt, suggesting the absence of any conformational transition. However, in 0.02 M $Mg(ClO_4)_2$, where poly(U) was shown to

exist in an ordered state below 5°C (Lipsett, 1960), the ORD curve was sensitive to temperature (Ts'o et al., 1966). Wolfe et al. (1969) reported that at room temperature, poly(U) had strong CD bands between 300 and 190 nm. In moderate salt concentration (0.5 M CsCl), the CD of poly(U) showed temperature dependence, which was independent of the polymer concentration (Thrierr et al., 1971).

Brown (1966) and Millar and Mackenzie (1970) reported that the molecular weight of poly(U) at room temperature determined by sedimentation equilibrium increased with ionic strength, indicating a salt-dependent aggregation. They also observed an increase in molecular weight upon lowering the temperature approaching 0°C, and whose magnitude depended on the nature and concentration of salt used. Contradictory to Brown's work (1966) as well as to that by Millar and Mackenzie (1970), Thrierr et al. (1971) found that poly(U) in 0.5 M CsCl had constant molecular weight as determined by light scattering, both above and below the T_m. Thus, it was concluded that the transition leading from disorder to order, the decrease in UV absorptivity or change in CD spectrum, reflected either an increase in stacking of single-stranded poly(U) or a folding of the polymer on itself (Thrierr et al., 1971). Like poly(dAT), alternating copolymer of deoxyribonucleotides of adenine and thymine (Inman & Baldwin, 1962), the intrinsic viscosity of poly(U) reached a minimum at its T_m (Thrierr & Leng, 1969; Millar & Mackenzie, 1970; Thrierr et al., 1971). This finding prompted them to propose that poly(U) at low temperature, had the structure similar to poly(dAT): a single hairpin form resulting from the folding of the molecule upon itself (Thrierr et al., 1971), at least in the absence

of Mg^{+2} ion (Millar & Mackenzie, 1970).

It is evident from the foregoing brief survey of the literature that the conformation of polyribonucleotides has been extensively investigated under various experimental conditions. But some problems remain to be resolved. In spite of voluminous amount of work on the ORD, and, to a lesser extent, the CD, of these polymers, there are both quantitative and qualitative discrepancies in the literature. Occasionally, poor experimental resolution has obscured subtle fine features of the ORD and the CD spectra, which have an important bearing on the conformation of the polymers. It is known that the conformation of poly(A), poly(G), poly(C) and poly(U) can be changed merely by adjusting the pH or temperature of the polymer solution. Yet, the relation of conformational change, such as the formation of double-stranded helices or the dissociation of multiple-stranded aggregates, to the precise size and shape of these polymers has not been studied in detail. Structural findings in X-ray studies of polynucleotide fibers drawn from the concentrated solution are often applied to dilute solution studies. This is based on the assumption, that the structure of polynucleotides is identical in solid state and in solution, which is easily questionable.

The objective of this work is to determine both the conformation and the molecular weight of synthetic polyribonucleotides with respect to changes in pH and temperature. Our experimental method will utilize both optical properties (ORD, CD and UV absorption) and hydrodynamic properties (viscosity and sedimentation) of these polymers in dilute solutions.

II. EXPERIMENTAL

A. Materials

Synthetic polyribonucleotides, poly(A), poly(G), poly(C) and poly(U) were purchased from both Miles Laboratories, Inc., and Schwarz BioResearch, Inc. Sodium chloride, potassium fluoride and ethyl ether were obtained from Mallinckrodt Chemical works, liquefied phenol was from Matheson Coleman and Bell Manufacturing Chemists, sodium cacodylate was from Sigma Chemical Company, sodium perchlorate was from G. Frederick Smith Chemical Company, citric acid and sodium citrate were from Allied Chemical, B & A General Chemical Division, Sephadex G-200 (Lot 1680) was from Pharmacia Fine Chemicals, Inc., Bio-Gel A-15 m was from Bio-Rad Laboratories, urea (ultra pure) was from Mann Research Laboratories, sucrose, was from National Bureau of Standards and d-10-camphorsulfonic acid was from Eastman Organic Chemicals. Water was double-distilled.

B. Methods

1. Preparation of Solutions

The polyribonucleotides were deproteinized by phenol extraction (Saito & Muira, 1963). A concentrated solution of the polymer (10 mg/ml) in 0.1 M NaCl containing 0.01 M EDTA (pH 7) was stirred with an equal volume of liquefied phenol saturated with water. The aqueous layer was collected after centrifugation and the procedure was repeated until there was no precipitate at the phenol-water interface. The polyribonucleotide was precipitated from the aqueous phase by the addition of cold ethanol, thoroughly washed with cold ethanol and ether, and then dried under vacuum. The polymer was then stored at -20°C.

Unless otherwise stated, polyribonucleotide solutions were prepared by dissolving the deproteinized polymer in 0.1 M NaCl (pH 7) with stirring. The solutions were then dialyzed exhaustively against appropriate buffer at 4°C. The buffers used were: solvent A (0.08 M NaCl and 0.02 M sodium citrate plus citric acid), solvent B (0.01 M sodium citrate-HCl buffer), solvent C (0.1 M NaCl plus 0.05 M cacodylate buffer) and solvent D (0.05 M KF and 0.02 M sodium citrate plus citric acid). In order to remove any contaminants and nucleases, the dialysis tubing had been successively boiled for hours in water, 10^{-3} M EDTA, 10^{-3} M HCl and water again. Solvents were filtered through Millipore filter type PH (pore size = 0.3 μ) before use and polyribonucleotide solutions through type HA (pore size = 0.45 μ) or SM (5 μ) or SC (8 μ). The pH of the solutions was measured on a Radiometer 25 pH meter equipped with expanded scale and combined electrode, which had been calibrated against standard buffers from Fisher or Beckman at pH 4, 5, 7 or 10.

The polyribonucleotide concentrations were determined spectrophotometrically with a Zeiss PMQII spectrophotometer using molar absorptivities per nucleotide residue as listed in Table I. These values were either determined in this laboratory on the basis of phosphorus analysis (Allen, 1940; Chen et al., 1956) or taken from the literature.

2. Optical Studies

a. Ultraviolet Absorption

The strong UV absorption of the purine and pyrimidine bases of polynucleotides and its sensitivity to structural changes have made UV spectroscopy one of the popular techniques in studying nucleic acids. A hyperchromic effect is observed when the bases are unstacked by

Table I

Molar Absorptivities of Polyribonucleotides

Material	Solvent, pH	λ_{max} (nm)	$\varepsilon \times 10^{-3}$
Poly(A), (S: 6902)[a]	Solvent A[b], pH 7.0	257	10.1[c]
Poly(G), (M: 11-06-314)[d]	0.01 M Sodium Citrate-HCl Buffer, pH 7.5	253	10.3
"	0.1 M NaCl, pH 6.5	253	10.9
Poly(G), (M: 11-08-314)	0.1 M NaCl-0.05 M Sodium Cacodylate Buffer, pH 7.0	253	9.8
Poly(C), (S: 6701)	Solvent A, pH 6.2	268	6.2
"	Solvent A, pH 4.8	275	7.1
Poly(U), (S: 7001)	0.1 M NaCl, pH 7.0	260	9.1[e]
"	1.0 M KF, pH 7.0	260	8.7

[a] S = Schwarz BioResearch, Inc.

[b] Solvent A = 0.08 M NaCl and 0.02 M Sodium Citrate plus Citric Acid

[c] From Adler *et al.* (1969)

[d] M = Miles Laboratories, Inc.

[e] From Simpkins *et al.* (1967)

heating or changes in pH or other means. Experimentally, the absorbance is expressed in terms of molar absorptivity (or molar extinction coefficient) by the Beer-Lambert Law:

$$\varepsilon = \log (I_o/I)/c\ell \qquad (1)$$

where I_o and I are the intensities of the incident and transmitted lights, c is the concentration in moles per liter and ℓ is the light path in centimeters. We used a Zeiss PMQII spectrophotometer and all measurements were done at room temperature unless stated otherwise.

b. Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD)

ORD and CD share a common origin, namely, optical activity. They are sensitive tools for studying the conformation of nucleic acids in solution (Yang & Samejima, 1969; Brahms & Brahms, 1970; Tinoco & Cantor, 1970).

Plane polarized light may be regarded as the resultant of right and left circularly polarized components of equal magnitude at the same frequency. On passing through an optically active medium, the two components will be absorbed differently by the optically active substance as well as exhibiting different refractive indices. The difference in absorbance is called circular dichroism ($A_L - A_R$) and that in the refractive indices is circular birefrigence ($n_L - n_R$). With circular birefrigence the right and left components will have different velocities which result in a phase difference upon recombination and the plane of polarization of the emergent light will be rotated through an angle, α. Quantitatively we have

$$\alpha \text{ (deg)} = 180\ell(n_L - n_R)/\lambda \qquad (2)$$

where α is the optical rotation at wavelength λ, and ℓ the path length of the medium. In the region of an optically active band where $A_L \neq A_R$, the transmitted light is no longer plane polarized but becomes elliptically polarized. The major axis of the ellipse is the sum of the amplitudes of the right and left circularly polarized components; the minor axis is their difference. The ellipticity, Ψ, is defined as the arctangent of the ratio of the minor axis of the ellipse to the major axis. It can be shown that (Yang, 1968):

$$\Psi \text{ (deg)} = 33(A_L - A_R) \qquad (3)$$

An anomalous ORD and CD associated with an optically active band is called a Cotton effect. Multiple Cotton effects are characteristic of helical polynucleotides with stacked bases (Warshaw et al., 1965; Sarkar & Yang, 1965a; Michelson et al., 1966).

The ORD is expressed as the mean residue rotation, [m], which is defined as:

$$[m] = 100\ \alpha/c\ell \qquad (4)$$

where α is the observed rotation in degrees, ℓ the pathlength in centimeters and c the concentration in moles of mean residue per liter; thus the unit of [m] is deg • cm^2/decimole. The CD is expressed as the mean residue ellipticity [θ], from the relation:

$$[\theta] = 100\ \Psi/c\ell \qquad (5)$$

where Ψ is the measured ellipticity in degrees and the dimension for [θ] is identical with that for [m].

The ORD spectra were measured on a Cary 60 recording spectropolarimeter and the CD on a Durrum Jasco J-10 recording spectropolorimeter, both under constant nitrogen flush. Specially designed thermostable cell holders and jackets were installed in both instruments. Most of the spectra were measured at 25°C unless stated otherwise. The temperature above 15°C of the solutions was regulated by a Haake constant-temperature circulator. For lower temperatures, an Aminco special refrigerated bath, a YSI thermistemp (model 71) temperature controller and a pump circulating mixture of glycerol and water were used. The temperature of the solution was monitored by a Leeds and Northrup millivolt potentiometer with a Cu-Constantan thermocouple attached to the block in the sample compartment. The solution at each temperature was allowed to equilibrate for 15-20 minutes. No corrections were made for volume variations with temperature. The absorbance of the solution was adjusted to keep the voltage of the instrument below 400 volts. Fused cylindrical silica cells (Pyrocell S-18-260) with pathlength of 1 to 25 mm were used for all measurements. Cells under 10 mm were calibrated with d-10-camphorsulfonic acid in H_2O using $\varepsilon_L - \varepsilon_R = 2.20$ at 290.5 nm (Cassim and Yang, 1969) or with a freshly prepared sucrose solution of the National Bureau of Standards grade with $[\alpha]_{589.3} = 66.45$ (Lowry, 1935).

3. Gel Chromatography

Sephadex G-200 was allowed to swell in water (20 gm/l) for 3 days. A column of 110 x 0.8 cm was packed at room temperature with 15 cm operating pressure at a rate of 2 to 2.5 min/drop. The column was

equilibrated with appropriate buffer for 1.5 days. Suspension of swollen agarose was also packed in a column of 60 x 0.8 cm. The polynucleotide solution was chromatographed at room temperature. Fractions of 0.6 ml were collected at flow rates varying from 50 to 100 seconds per drop for different preparations with an automatic fraction collector.

4. Hydrodynamic Studies

a. Sedimentation Velocity

Sedimentation velocity experiment permits the direct measurement of the sedimentation coefficient which is related to the size and the shape of the sedimenting molecules. The sedimentation coefficient is defined as the sedimentation velocity divided by the centrifugal acceleration that produces it (Schachman, 1959).

$$s = (dr/dt)/\omega^2 r = 2.303(d \log r/dt)/60\omega^2 \qquad (6)$$

where s is the sedimentation coefficient in seconds, and the quantity, 1×10^{-13} sec is called a Svedberg, denoted by S; r is the distance from the center of rotation to the boundary in centimeters; ω is the angular velocity in radians per sec, and t and t' are the time in seconds and minutes. Thus a plot of log r versus time will give a straight line for a homogeneous sample in an ideal solution. A plot that is concave upward indicates polydispersity while non-ideality results in a curve that is concave downward. Thus, non-ideal polydisperse samples may fortuitously result in a straight line. The sedimentation coefficients are generally reported as $s_{20,w}$, the value the materials would have in a solvent with the density and viscosity of water at 20°C. Corrections of

the observed sedimentation coefficient, s_{obs}, to this standard state are made according to the equation (Svedberg and Pedersen, 1940).

$$s_{20,w} = s_{obs}(\eta_t/\eta_{20})(\eta/\eta_o)[(1-\bar{v}\rho_{20,w})/(1-\bar{v}\rho_t)] \qquad (7)$$

where (η_t/η_{20}) is the principal correction factor corresponding to the viscosity of water at t° relative to that at 20°C,

(η/η_o) is the relative viscosity of the solvent to that of water,

$\rho_{20,w}$ is the density of water at 20°C,

ρ_t is the density of solvent at t°,

$\bar{v}$ is the partial specific volume of the solute.

The concentration dependence of the sedimentation coefficient can be fitted with Equation (8):

$$1/s = 1/s^\circ + k\, c/s^\circ \qquad (8)$$

where s° and s are the sedimentation coefficients at infinite dilution and concentration c, k is a constant which is generally found to be positive; thus, s usually decreases with increasing concentration. This concentration dependence of sedimentation coefficient has the effect of sharpening the boundary, especially for more rigid and extended polynucleotides. Thus, it may mask the existence of polydispersity.

Experimentally, schlieren optics, which monitors the refractive index gradient, is widely used for locating the moving boundary of the sedimenting solute particles. But relatively high concentrations of the sample are required for best results. The strong UV absorption

of all polynucleotides makes it possible to use the absorption optical system. This method permits measurements with concentration about 1/10 to 1/100 of that required for the refractometric methods, thereby circumventing the extrapolation to zero concentration. There are two UV absorption optical systems: photographic and photoelectric scanning. The former one has the drawbacks of possible non-uniform blackening of the film and the lack of direct viewing. On the other hand, the split-beam photoelectric scanner allows direct viewing of the difference in absorbance between the sample and reference sectors of the ultracentrifuge cell at any radial position.

b. Diffusion

The diffusion coefficient, D, is a measure of the mass of solute transported across a plane of known cross-section in a given period of time under the influence of a driving force. In a solution, the driving force is essentially the concentration gradient (Schachman, 1957). The diffusion coefficient is related to the frictional coefficient, f, as:

$$D = RT \left[1 + c(\partial \ln y/\partial c)_{T,p}\right] /Nf \qquad (9)$$

where R is the gas constant, N the Avogadro's number, T the absolute temperature, c the concentration, and y the activity coefficient of solute. The diffusion coefficient at infinite dilution, D°, can be obtained by measuring D at several different concentrations and extrapolating it to zero concentration,

$$D^\circ = RT/Nf = kT/f \qquad (10)$$

where k is the Boltzmann's constant. Experimentally, D is related to the concentration gradient, dc/dr, as:

$$dc/dr = -[c_o/2(\pi Dt)^{1/2}]\, e^{-r^2/4Dt} \qquad (11)$$

where c_o is the initial concentration and t the time. The concentration gradient is proportional to the refractive index gradient, dn/dr; thus, it can be determined with schlieren optics as in sedimentation velocity experiments. It is seen from Equation (11) that the maximum value of $|dc/dr|$ is $c_o/[2(\pi Dt)^{1/2}]$ at r = 0. The maximum height, H, of the refractive index gradient *versus* r curve is $kc_o/[2(\pi Dt)^{1/2}]$, where k is a constant. The area, A, of the refractive index gradient curve is kc_o. Thus, we have

$$A/H = (4\pi Dt)^{1/2} \qquad (12)$$

Therefore, a plot of $(A/H)^2$ *versus* t would yield a straight line, the slope of which is $4\pi D$. As in the case of sedimentation coefficient, D should be corrected to the standard condition in water at 20°C by the relationship:

$$D_{20,w} = D[293/(273 + t)]\, (\eta_t/\eta_{20})\, (\eta/\eta_o) \qquad (13)$$

here t refers to temperature in centigrade. With s° and D° known, the molecular weight, M, can be determined from the Svedberg equation:

$$M = RTs°/D°(1-\bar{v}\rho) = Nfs°/(1-\bar{v}\rho) \qquad (14)$$

c. Sedimentation Equilibrium

The condition for sedimentation equilibrium is achieved when the rate of transporting solute due to sedimentation is balanced by the reverse transport from diffusion. Thermodynamically, the total potential which is the sum of the chemical potential and the centrifugal potential, is independent of time and position in the ultracentrifuge cell when the equilibrium condition has been attained. The fundamental equation for sedimentation equilibrium is (Van Holde, 1967)

$$[dc(r)/dr]/c(r) = [M(1-\bar{v}\rho)\omega^2 r] /RT \qquad (15)$$

where c(r) is the concentration at point r from the axis of rotation. The integration from the meniscus, m, to point, r, gives the concentration distribution throughout the cell,

$$\ln [c(r)/c(m)] = [M(1-\bar{v}\rho)\omega^2/RT] (r^2-m^2)/2 \qquad (16)$$

Equation (15) applies only to a homogeneous, ideal, two-component system. Thus, the plot of ln c(r) versus r^2 gives a straight line whose slope is proportional to the molecular weight. A plot which is concave upward indicates polydispersity and concave downward indicates non-ideality of the solution. For a polydisperse system, the molecular weight is weight-average, M_w, which is:

$$M_w = \Sigma N_i M_i^2 / \Sigma N_i M_i \qquad (17)$$

where N_i is the number of moles of the i-th species having a molecular weight M_i.

The photoelectric scanning system allows direct recording of the absorbance and thereby concentration, c, versus the radial position, r. Solutions having high molar absorptivity such as polyribonucleotides can be studied at extremely low concentration by using the 265-nm absorption band. For practical purpose, it can be considered to be approaching the ideal condition and the data can be directly used to calculate the molecular weight according to Equation (16). The Rayleigh interferometric system measures the displacement of fringes due to difference in concentration, and thereby refractive indices between the solution and solvent. Two techniques have been developed to determine the molecular weight, using the Rayleigh optics. One is known variously as the "high-speed method", the "Yphantis method", or the "meniscus depletion method", in which the ultracentrifuge is run at such a high speed that the solute concentration at the meniscus, c(m), is effectively zero (Yphantis, 1964). Thus, the displacement of fringes at various radial position, r, measures directly the concentration at that point which enables us to calculate the molecular weight by Equation (16). The second one is the "low-speed" method. From the displacement of fringes we obtained the concentration difference between the bottom and meniscus, c(b)-c(m). It can be shown (Williams et al., 1958) that

$$[c(b)-c(m)]/c_o = M(1-\bar{v}\rho)\omega^2 (b^2-m^2)/2RT \qquad (18)$$

where c_o is the initial concentration and b and m are the distances from the axis of rotation to the bottom and meniscus of the cell. Equation (18) provides another method for determining the molecular weight.

Since the Rayleigh optics system demands the use of moderately

concentrated solutions, accordingly, Equation (15) must be modified to take into consideration the non-ideality of the solution:

$$[dc(r)/dr]/c(r) = [M(1-\bar{v}\rho)\omega^2 r]/RT[1 + c(\partial \ln y/\partial c)] \quad (19)$$

Thus, the apparent molecular weight, M_{app}, at any finite concentration can be written as:

$$1/M_{app} = [1 + c(\partial \ln y)/\partial c]/M \quad (20)$$

By plotting $1/M_{app}$ against c, we obtain the true molecular weight from the reciprocal of the intercept on the ordinate.

All the sedimentation and diffusion experiments were carried out in two Spinco model-E ultracentrifuges, one equipped with a monochromator and photographic UV absorption optics and the other with a monochromator, photoelectric scanner and multiplexer. The wavelength of light was set at 265 nm. The 12-mm single- or double-sector cells with Kel-F centerpieces were routinely used. AN-D two-hole aluminum rotor and AN-F four-hole aluminum rotor were used for speeds higher than 10,000 rpm and the heavy AN-J four-hole aluminum rotor was used for speeds lower than 10,000 rpm.

For sedimentation velocity experiments, we used either schlieren optics, photographic UV absorption optics or photoelectric scanner and multiplexer. With schlieren optics single-sector cells with quartz windows were employed; for photographic UV absorption optics, sapphire windows were used instead. When two solutions were run simultaneously, 1° positive wedge window was used in one of the cells. With the photoelectric scanner, double-sector cells with sapphire

windows and the special wide aperture window holders were used along with the special scanner counter-balance. Routinely three double-sector cells were run at the same time through the use of the multiplexer accessory. The schlieren patterns were recorded on Kodak metallographic plates and analyzed on a Nikon microcomparater. Kodak professional films were used for the photographic UV absorption optics and the films were scanned on a Zeiss PMQII spectrophotometer equipped with a Vicon gel-scanner and a recorder. With the photoelectric scanner, tracings of absorbance versus radial position were directly recorded and a 10-inch Leeds-Northrup recorder was used with the scanner.

Diffusion experiments were carried out with the schlieren optics and a double-sector cell with a capillary synthetic boundary centerpiece was used. One sector of the cell contained 0.15 ml of solution and the other one was filled with the solvent dialyzate. Upon acceleration the excess solvent passed through the capillary and formed a synthetic boundary over the polynucleotide solution. A heavy AN-J rotor was routinely used at 4,609 rpm. The area under the schlieren peak was determined by trapezoidal approximation and the maximum height of the schlieren peak was also measured.

Sedimentation equilibrium experiments were carried out either with Rayleigh interference optics or with the photoelectric scanner and multiplexer. With interference optics a double-sector cell with sapphire windows and the Yphantis 6-channel centerpiece or double-sector centerpiece was employed along with the interference counterbalance. The Rayleigh patterns were recorded on a Kodak spectroscopic II-G plates and were read on a Nikon Microcomparator. For the photoelectric scanner, the double-sector scanner cell with sapphire windows and the special scanner counterbalance were used. Cells were filled with 0.01

ml of FC-43 fluorocarbon oil and 0.14 ml of solvent dialyzate in one sector and 0.02 ml oil and 0.1 ml solution in the other so that the solution column was about 3 mm in height. The oil was added to each compartment of the double-sector cell in order to produce a transparent region at the cell bottom which permits accurate measurements of the fringes (Rayleigh optics) or absorbance (photoelectric scanner) throughout the solution column. In the expanded traces the 3 mm column of the solution appeared as 5 cm on the final traces.

When the Yphantis "high-speed" method was used, the operating speed was so chosen that the quantity $\omega^2 M(1-\bar{v}\rho)/RT \tilde{=} 5$ when the solution column was 3 mm in height. In the "low-speed" method, a concentration ratio of three or four to one between the ends of the solution column was obtained. The durations of the experiments were about 20 hours for the "high-speed" method and 48-72 hours for the "low-speed" one. The attainment of equilibrium was checked by superposition of fringes or tracings taken at intervals of several hours.

d. Viscosity

Viscosity measures the frictional resistance of fluids to flow. The addition of macromolecules to the fluid increases its viscosity, η_o, to a new value, η, since the macromolecules disturb the flow lines of the fluid. In 1906, Einstein found that for rigid, spherical particles in dilute solution,

$$\eta/\eta_o = 1 + 2.5\phi \qquad (21)$$

where ϕ is the volume fraction of the solution occupied by solute particles. By tradition, the viscosity data are expressed in terms of

intrinsic viscosity, [η], which is defined as:

$$[\eta] = \lim_{c \to o} \eta_{sp}/c = \lim_{c \to o} (\eta - \eta_o) / \eta_o c \qquad (22)$$

where η_{sp} is termed the specific viscosity and c is the concentration of the solute in gm/dl and equals to 100 ϕ/v_{sp} (v_{sp} is the specific volume of the solute in solution). Thus, Equation (21) for spheres can be written as:

$$[\eta] = 2.5\ v_{sp}/100 \qquad (23)$$

Simha (1940) derived an equation for the viscosities of ellipsoids of revolution:

$$[\eta] = \nu v_{sp}/100 \qquad ([\eta] \text{ in dl/gm}) \qquad (24)$$

where ν, the viscosity increment, is a function of axial ratio, p = a/b, (a and b are the semimajor and semiminor axes of the ellipsoid of revolution).

Many empirical equations have been proposed to express the concentration dependence of viscosity of macromolecules. For dilute solutions, the most commonly used one is the Huggins equation (Huggins, 1942),

$$\eta_{sp}/c = [\eta] + k' [\eta]^2 c \qquad (25)$$

where k' is a factor related to solute-solute and solute-solvent interaction (Yang, 1961; Bradbury, 1970). Thus, from a plot of η_{sp}/c versus concentration, the intrinsic viscosity, [η], and k' can be

obtained from the intercept and the slope, respectively.

Oncley (1941) proposed that v_{sp} in Equation (24) be replaced by the experimentally measurable partial specific volume, $\bar{v}$, and ν by $\nu_h (1 + w/\bar{v}\rho)$ where w is water of hydration in grams per gram of the solute and ρ the density of the solvent. By assuming a reasonable value for w, one can determine the viscosity increment, ν, which in turn provides a rough estimate of the axial ratio of the ellipsoid of revolution (usually a prolate rather than an oblate ellipsoid is assumed in such calculations). The Oncley treatment has been criticized on various grounds (Scheraga & Mandelkern, 1953). In particular any single hydrodynamic property, such as $[\eta]$ or s°, can not give a definite solution of the two unknowns, the axial ratio and the specific volume of the ellipsoid of revolution.

An alternative approach to this problem was to combine two hydrodynamic properties, which in principle can solve the two unknowns. Scheraga & Mandelkern (1953) introduced the notion of hydrodynamically equivalent ellipsoid of revolution characterized by the effective volume, Ve and axial ratio, p. Equation (24) can be written as:

$$[\eta] = \nu \, N \, Ve/100 \, M \tag{26}$$

From Stokes' law,

$$f_\circ = 6 \, \pi \, \eta_\circ \, (3 \, Ve/4 \, \pi)^{1/3} \tag{27}$$

where $f_\circ$ is the translational frictional coefficient of a sphere having the same volume, Ve, as the hypothetical (impermeable, unsolvated and rigid) ellipsoid with a translational frictional coefficient, f. By

writing $f_o/f = F$ and combining Equations (26) and (27) with the elimination of Ve, we obtain,

$$\beta \equiv \eta_o [\eta]^{1/3} M^{1/3}/f = (N/16200\pi^2)^{1/3} F\nu^{1/3} \qquad (28)$$

where f can be determined from sedimentation coefficient [Eq. (14)] or diffusion coefficient [Eq. (10)]. The right-hand side of Equation (28) is a function of the axial ratio for prolate and oblate ellipsoids of revolution only. The relation between the axial ratio and viscosity increment, ν, has been evaluated by Simha (1940), and that between axial ratio and F by Perrin (1936). It is noted that for a given axial ratio the β-value is different for the prolate and oblate ellipsoids of revolution. However, the prolate model is used in most cases.

The β-function is rather insensitive to axial ratio of the particle. However, its very insensitivity to shape provides a new method for estimating the molecular weight of the particles. With measured $[\eta]$ and f (obtained from s° or D°), a decision about the particle shape (prolate or oblate) is made, and an approximate value of p is chosen (with the Oncley treatment, for instance). This in terms defines the β-values. The molecular weight as calculated from Equation (28) is usually accurate to ± 10%. An equation similar to the β-function has been derived for flexible coils (Mandelkern & Flory, 1952; Mandelkern *et al.*, 1952). The constant, $\Phi^{1/3}P^{-1}$, that corresponds to the β-value is found experimentally to be 2.5×10^6.

Viscosity was measured in a suspension-type Ubbelohde two-bulb viscometer. The temperature of the bath was maintained at 25 ± 0.05°C unless otherwise mentioned. Two viscometers were used with flow time for water of about 1,000 and 270 seconds for the lower bulb and 700

and 200 seconds for the upper bulb, respectively. At least three measurements were made at each concentration and the results averaged. The maximum range of variations was one second.

III. RESULTS

A. Polyriboadenylic Acid

1. Optical Studies

Figure 2 shows the UV absorption spectra of poly(A) in solvent A (0.08 M NaCl, 0.02 M sodium citrate plus citric acid). Lowering the pH from 6.5 to 5.8 causes the absorption maximum to shift from 257 to 253 nm with a marked decrease in absorbance. Also the spectrum shows a slight red-shift near 290 nm. At pH 5.0, the whole absorption spectrum appears to be further blue-shifted by 1 nm and it remains unchanged even at pH 4.2. Thus, poly(A) has nearly the same UV absorption spectra at pH 5.8 and 5.0 or lower which are very different from that at pH 6.5. Adler et al. (1969) also observed a 4-nm blue shift when the pH was lowered from 8.5 to 5.81, although no details were given in their paper.

Figures 3 and 4 show the ORD and CD of poly(A) in solvent A at various pHs. The solid lines represent the spectra at pH 6.1, where poly(A) is single-stranded with the bases partially stacked, and at pH 4.2, where it is believed to be a double-stranded helix. This shift in ORD and CD agrees with that in the UV absorption spectrum. As the pH is lowered to pH 5.86, the first peak of the ORD is slightly red-shifted and its magnitude increases. At pH 5.81, the peak is further red-shifted and the magnitude is very much enhanced; while the trough is blue-shifted and the shoulder of the neutral form at 230 nm disappears. The ORD of pH 5.4 shows two shoulders located on both sides of the peak of that at pH 5.81 with slightly increased magnitude. Adler et al. (1969) reported a transition from pH 5.91 to 5.81 with increased magnitude and suggested that the ORD at pH 5.81

Figure 2: UV absorption spectra of poly(A) at 25°C in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at pH 6.5, 5.8 and 5.0.

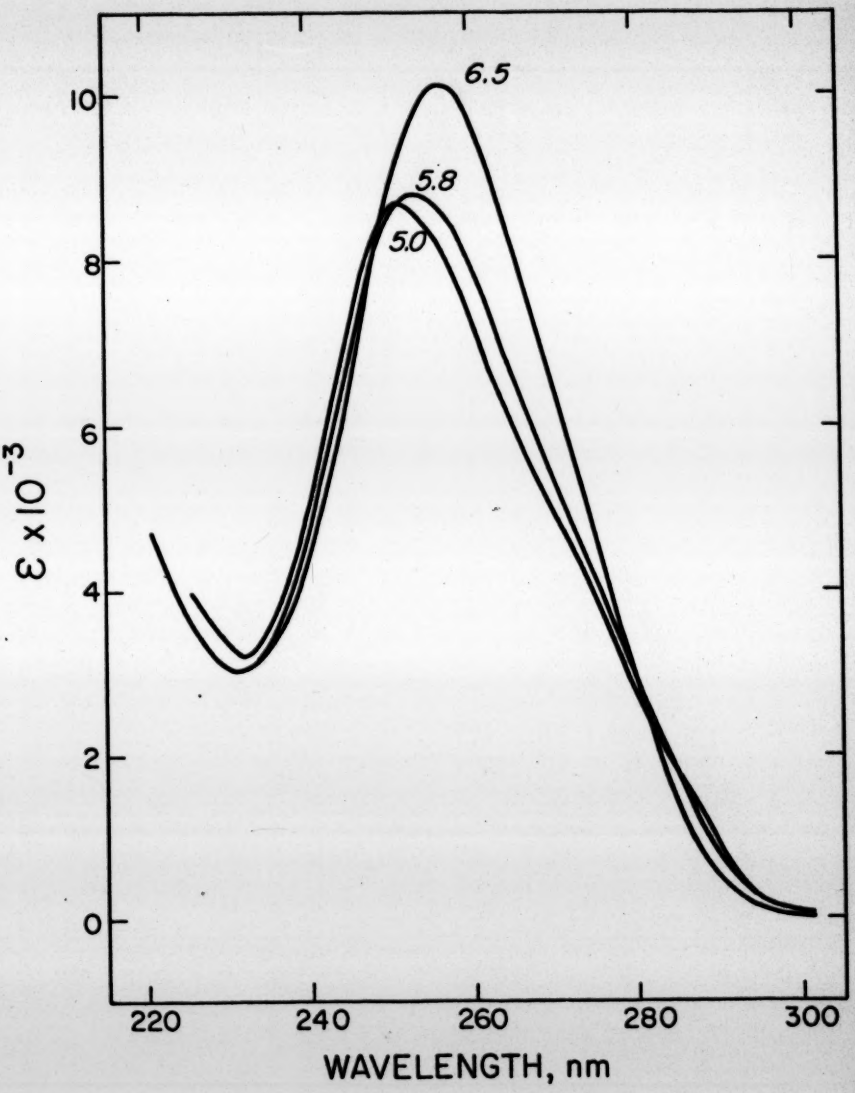

6.5
5.8
5.0
10
8
6
4
2
0
ε x10⁻³
220
240
260
280
300
WAVELENGTH, nm

Figure 3: ORD spectra of poly(A) at different pHs in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at 25°C. (Lot No. S: W-2065)

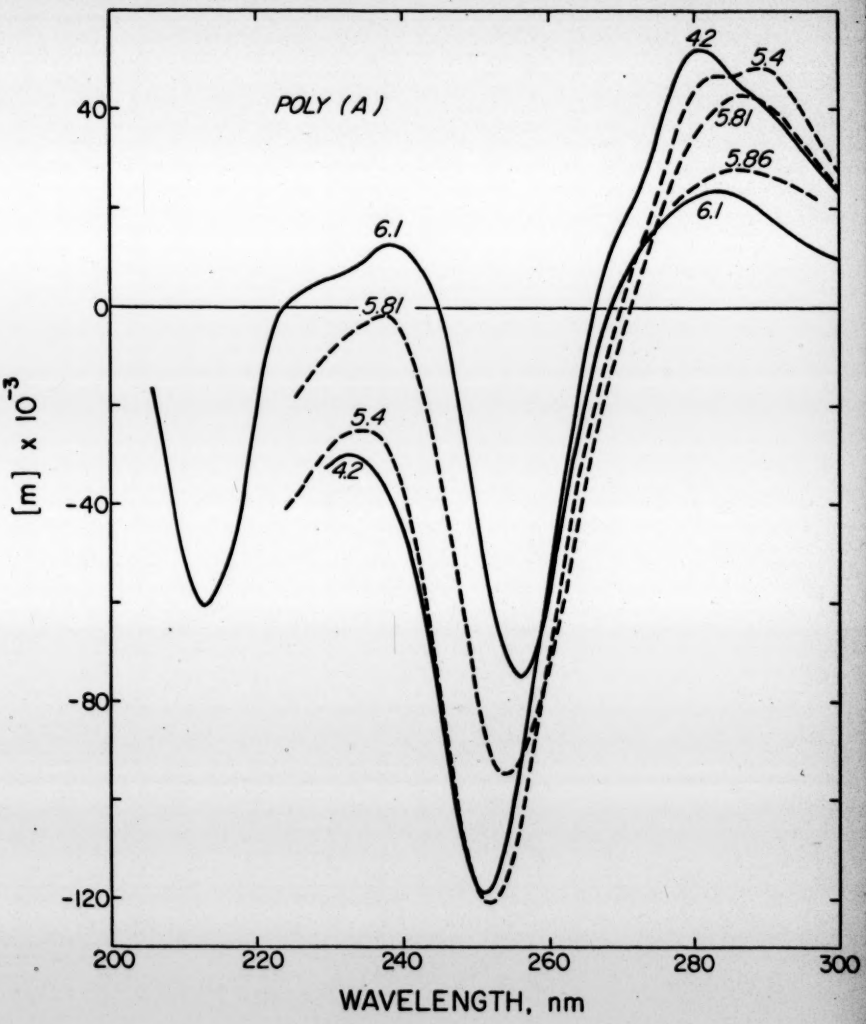
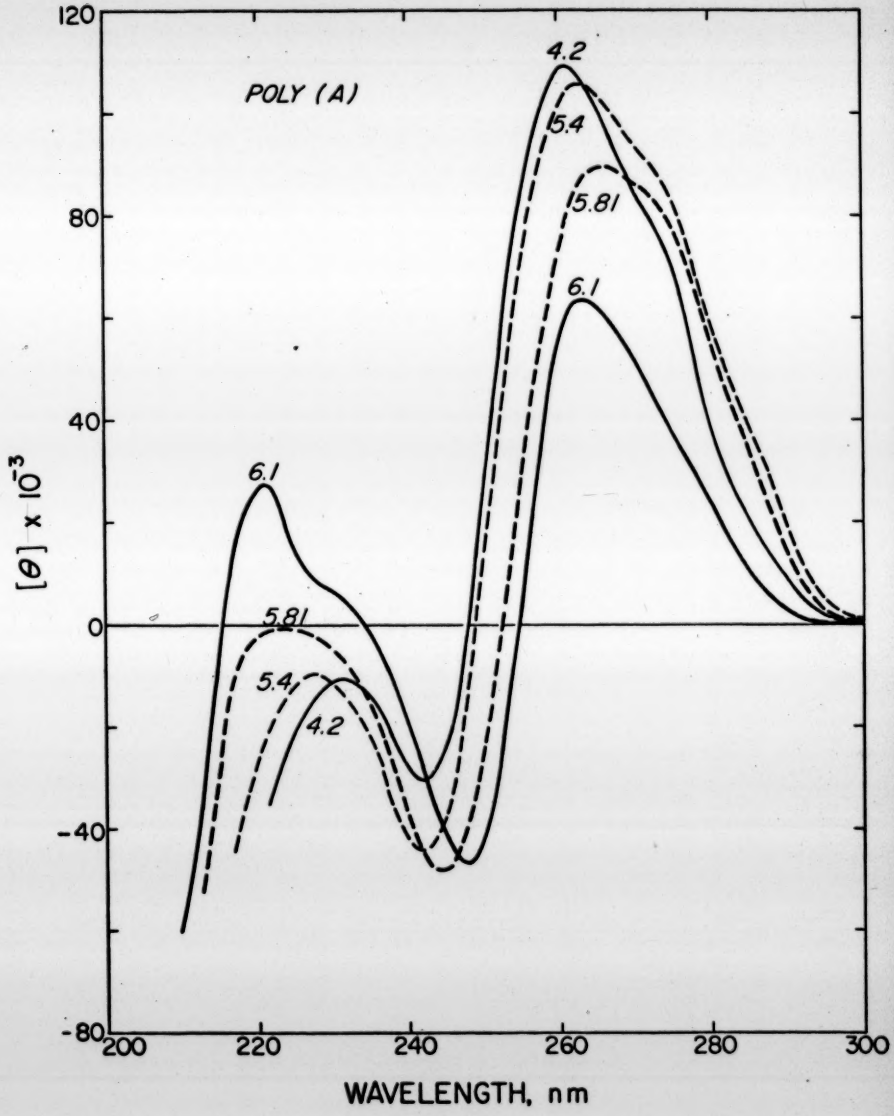

POLY (A)
4.2
5.4
5.81
5.86
6.1
6.1
5.81
5.4
4.2
40
0
-40
-80
-120
200
220
240
260
280
300
[m] x 10^-3
WAVELENGTH, nm

Figure 4: CD spectra of poly(A) at different pHs in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at 25°C. (Lot No. S: W-2065)

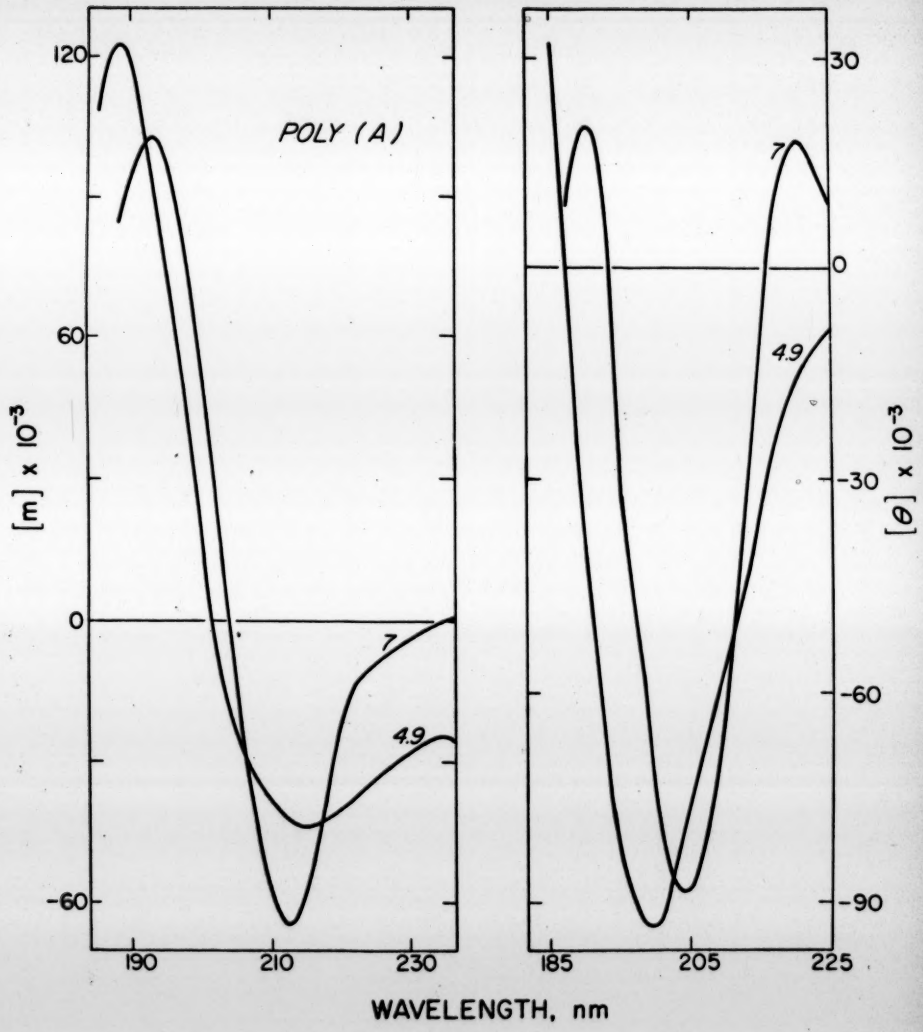

120
80
40
0
-40
-80
200
220
240
260
280
300
POLY (A)
4.2
5.4
5.81
6.1
6.1
5.81
5.4
4.2
[θ] x 10⁻³
WAVELENGTH, nm

was the acidic B form. They also observed a double positive peak at pH 5.0 whose magnitude was less than that at pH 5.81 and 4.0. This was designated as a mixture of the B and A forms of the acidic double-stranded helix of poly(A), although the difference in conformation between the two forms was not known. But we found that the magnitude of the twin peaks between pH 5.6 and 5.1 was always larger than that at pH 5.7-5.9 and smaller than that at pH 4.2. Moreover, our ORD spectrum at pH 5.0 resembled that at pH 4.2 rather than the double peak profile. (Previous work on acidic poly(A) at pH 4.85, 0.2 M Na^+ ion (Sarkar & Yang, 1965a) and pH 5.0, 0.05 M $NaClO_4$, 1×10^{-3} M HAc-NaAc (Ts'o et al., 1966) did not detect this feature of a "double positive Cotton effect".)

The CD of poly(A) at pH 6.1 indicates an apparent skewness near 280 nm and a shoulder at 230 nm (Bush & Scheraga, 1969). At pH 5.81, this "skewness" is largely red-shifted with a broadening of the positive band and an increase in its magnitude. Also the shoulder of pH 6.1 at 230 nm becomes one broad band indicating similar changes as in the ORD measurements. At pH 5.4, the positive band is further intensified with the extremum closer to that of pH 4.2 and a shoulder on the longer wavelength side. The "skewness" in the CD curve of poly(A) at neutral pH (Bush & Scheraga, 1969) or the "shoulder" at acidic pH (Brahms & Mommaerts, 1964) has been attributed to the contribution of an $n-\pi^*$ transition centered at 280 nm. This tentative assignment seems to find further support in the high-resolution absorption spectra of poly(A) (Brahms et al., 1969).

Below 230 nm, additional Cotton effects of poly(A) in both neutral and acidic solutions of 0.01 M KF were observed (Figure 5). The ORD at pH 7 shows a trough at 213 nm and a large peak at 193 nm which agree

Figure 5: ORD and CD spectra of poly(A) at 25°C in 0.01 M KF at pH 7 and 4.9 below 230 mμ. (Lot No. S: W-2065)


POLY (A)
7
4.9
120
60
0
-60
190
210
230
[m] x 10⁻³
30
0
-30
-60
-90
185
205
225
[θ] x 10⁻³
WAVELENGTH, nm

with that in 0.05 M $NaClO_4$ (pH 7) reported by Ts'o et al., (1966) only our magnitude is about 10% less. At pH 4.9, the trough and peak are located at 216 and 188 nm. The CD at pH 7 shows a large negative band at 204 nm as that in 0.1 M potassium phosphate salt (pH 7.2) reported by Wolfe et al. (1969) but our magnitude is about 30% as large. It also has a positive band at 190 nm. The CD at pH 4.9 shows a negative extremum near 200 nm and a large positive one below 185 nm which is hypsochromic to that at pH 7.

The Cotton effects of polynucleotides were reported to be dependent upon their previous history (Fasman et al., 1964) and pre-heating to 95-100°C for 5 to 10 min. could increase the magnitude of rotation or ellipticity extrema by about 30% (Adler et al., 1969; Davidson & Fasman, 1971). We have thus studied samples from Miles Laboratories Inc. (Lot No. 110748) and from Schwarz BioResearch, Inc. (Lot No. 6902 and w-2065); both of which, however, give almost the same result (Table II). We have carried out the same heating process and found that the absorbance at 257 nm of the solutions increased by about 5% after heating at 95-96°C for 5--10 min. The concentrations of the solution before and after heating were determined by ribose content (Schneider, 1957) and found to have the same changes as the absorbance at 257 nm. Our Cotton effects of poly(A) without heating are comparable to those heated (Adler et al., 1969; Davidson & Fasman, 1971). The difference may lie in the fact that Adler et al. (1969) adjusted the pH of the solution with 1 M HCl whereas we dialyzed the solution against the solvent of desired pH.

2. Hydrodynamic Studies

a. Viscosity

Table II

Cotton Effects of Poly(A)[a]

(in 0.08 M NaCl and 0.02 M Sodium Citrate plus Citric Acid at 25°C)

Solvent	ORD Extrema[b]						CD Extrema[b]					
pH (Lot No.)	λ_1	$[m]_1$	λ_2	$[m]_2$	λ_3	$[m]_3$	λ_1	$[\theta]_1$	λ_2	$[\theta]_2$	λ_3	$[\theta]_3$
7.0 (M: 110748)	284	24.8	256	-70.3	239	12.8	264	57.6	248	-44.2	221	27.5
6.1 (S: w-2065)	"	23.7	"	-70.9	"	12.4	263	63.3	247-8	-46.7	---	---
6.1 (S: 6902)	"	23.9	"	-74.9	"	11.1	---	---	---	---	---	---
5.92 (M: 110748)	"	32.0	"	-80.2	"	19.2	264	67.7	247	-49.7	220-1	25.3
5.81 (M: 110748)	287	45.4	254	-97.8	236	-5.3	266	84.1	244	-48.1	224	-1.1
5.81 (S: w-2069)	"	43.6	"	-95.0	238	-1.4	"	89.3	---	---	---	---
5.76 (S: 6902)	288	44.2	253	-104	236	-8.1	265	89.4	244	-51.1	---	---
5.4 (S: w-2065)	290	48.7										
	284	46.9	251	-118	234	-24.6	263	105	242	-46.8	227	-10.0
4.5 (S: 6902)	281	51.6	"	-117	---	---	261	112	241	-32.9	---	---
4.2 (S: 6902)	"	52.5	"	-119	232	-28.3	"	109	"	-29.2	230	-10.0

[a] [m] and [θ] in 10^3 deg cm^2/decimole and λ in nm

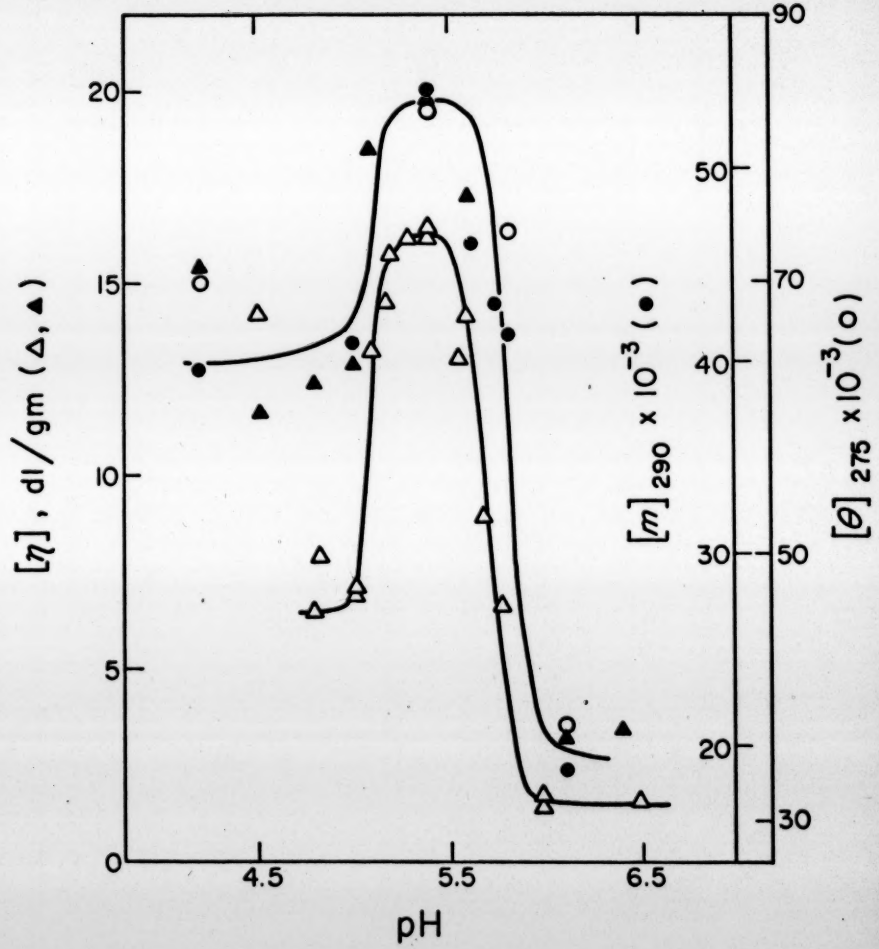
[b] Other extrema in 0.01 M KF: at pH 7; $[m]_{213} = -65.0$, $[m]_{193} = 103$, $[\theta]_{220} = 18.5$, $[\theta]_{204} = -88.0$, and $[\theta]_{190} = 20.0$; at pH 4.9, $[m]_{216} = -44.0$, $[m]_{188} = 122$, and $[\theta]_{200} = -93.0$

Figure 6 shows the changes in intrinsic viscosity of poly(A) with pH in acidic solution. Upon lowering the pH from 6.5, the intrinsic viscosity increases abruptly near the pK_a of the base (5.87 in 0.1 M NaCl according to Adler et al., 1969); it reaches a maximum plateau between pH 5.6 and 5.2, and then drops to a minimum value at pH 5.0-4.8. Two batches of sample (Lot No. S: 6902 & S: w-2065) from Schwarz BioResearch Inc. were studied and they demonstrated the same results. A viscometer with flow time for water of 200 and 300 seconds at 25°C for the upper and lower bulbs was used. The concentrations of solution used were so chosen that the difference in flow time between the solution and the solvent was between 40 and 150 seconds.

The solutions were filtered through Millipore filters before measurements. The extent of aggregation was judged by the decrease of absorbance of the solutions after filtration. The solutions of sample Lot No. S: 6902 were filtered through type SM (pore size, 5 μ) filter and those of Lot No. S: w-2065 at pH > 6.0 through HA (0.45 μ) filter, at 5.2 < pH < 6.0 through SM (5 μ) filter and at pH < 5.2 through SC (8 μ) filter. We found that by passing through SC (8 μ) filter, the absorbance of the solution decreased by about 5.4% at pH 4.8 and up to 50% at pH below 4.5. Thus complications due to aggregation at high concentration or low pHs made precise viscosity measurements difficult.

Along with the intrinsic viscosity, $[m]_{290}$ and $[\theta]_{275}$ are also plotted against pH in Figure 6 for comparison. Since the transitions are sharp, slight change in pH will shift the magnitude markedly, thus resulting in the scattering of experimental points. Nevertheless, both optical and hydrodynamic properties of poly(A) show a bell-shaped

Figure 6: Variations of the intrinsic viscosities and CD and ORD extrema of poly(A) with pH in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at 25°C. △ , $[\eta]$ of Lot No. S: 6902; ▲ , ○ , ● , $[\eta]$, $[\theta]_{275}$ and $[m]_{290}$ of Lot No. S: W-2065.


[η], dl/gm (△, ▲)
[m]290 x 10-3 (●)
[θ] 275 x 10-3 (○)
pH
0
5
10
15
20
4.5
5.5
6.5
20
30
30
40
50
50
70
90

curve with respect to pH. Adler et al. (1969) reported that the ratio of the twin peaks, $[m]_{280}/[m]_{287}$ first dropped sharply at pH 5.81 and then increased continuously with decreasing pH in the range of 5.8-4.0. They interpreted this as the gradual conversion of B form to A form. We found, however, that the ratio of these two peaks first decreased with pH, reached a plateau and then increased again at lower pH. The transitions occurred at the same pH range as those in Figure 6 (data not shown). Thus, our results suggest two transitions, one at pH 5.7-5.9 and the other at 5.0-5.2.

b. Sedimentation Velocity and Diffusion

Sedimentation (s) and diffusion (D) coefficients of poly(A) of Lot No. S: 6902 were determined by ultracentrifugation with schlieren optics. Figure 7 shows the concentration dependence of s_{20} and D_{20} in solvent A at pH 6.2. The s values of another sample (Lot No. S: w-2065) at different pHs were determined by ultracentrifugation using the photoelectric scanner at 50,740 rpm. All solutions were filtered through Millipore filters having the same pore sizes as those used in viscosity studies at different pHs. Since the concentrations used in the experiments with the scanner were of the order of 10^{-4} moles of nucleotides/liter, we did not study the concentration dependence of the sedimentation coefficient. Table III lists the s values of poly(A) at various pHs along with their intrinsic viscosities.

3. Determination of Molecular Weight

1. Sedimentation Equilibrium

Sedimentation equilibrium was carried out with the photoelectric scanner between 19 and 20°C. Since the concentrations used were very

Figure 7: Concentration dependence of s_{20} and D_{20} of poly(A) in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at pH 6.2 (Lot No. S: 6902)

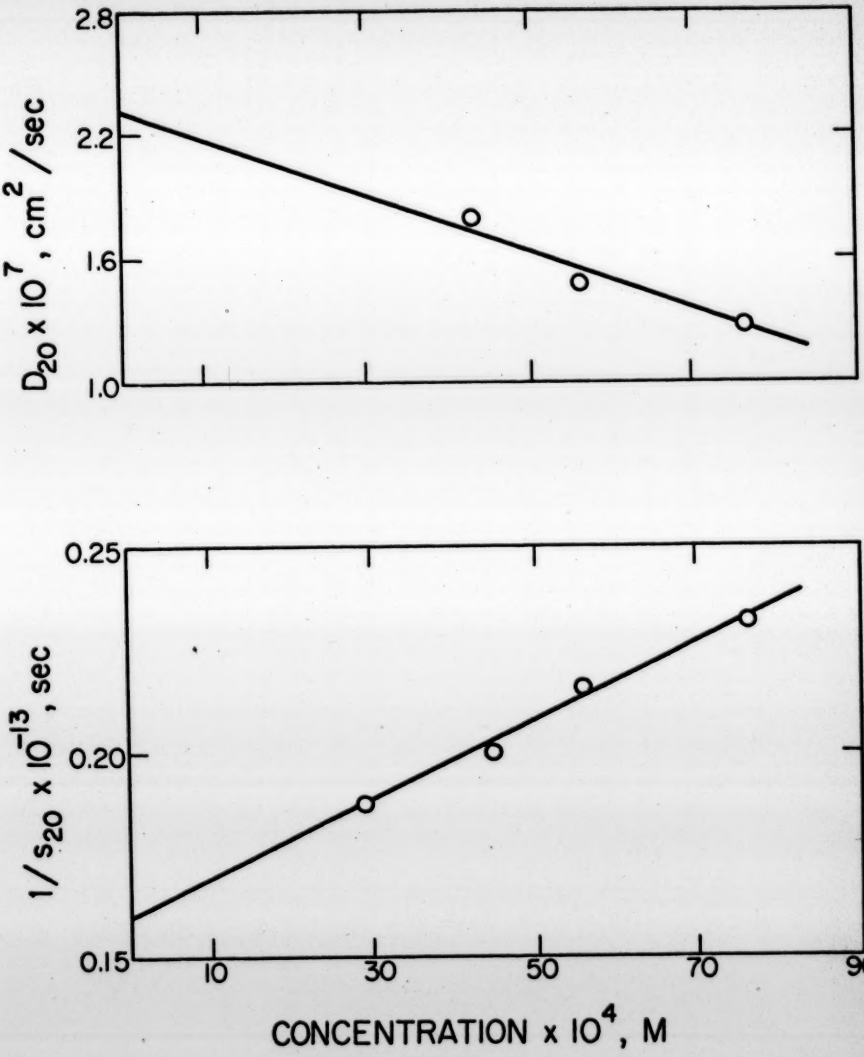

2.8
2.2
1.6
1.0
$D_{20} \times 10^{7}$, cm^2/sec
0.25
0.20
0.15
$1/s_{20} \times 10^{-13}$, sec
10
30
50
70
90
CONCENTRATION x 10^4, M

Table III

Hydrodynamic Properties and Molecular Weights of Poly(A)

Solvent pH	$[\eta]_{25}$ dl/gm	k'	β x 10^{-6}	$\Phi^{1/3} P^{-1}$ x 10^{-6}	s°_{20} S	s°_{25} S	D°_{20} x 10^{7}, cm^2/sec	M_{β} x 10^{-5}	$M_{\Phi^{1/3} P^{-1}}$ x 10^{-5}	M_{s-D} x 10^{-5}	$M_{sed.\ eq.}$ x 10^{-5}
S: w-6902											
6.2	1.5	0.3	3.04	2.5	6.3	(7.0)[a]	2.32	1.7	2.3	1.5	---
S: w-2065											
6.5	3.5	0.3	3.2	2.5	(7.6)	8.5	---	3.3	4.8	---	3.1
6.1	3.3	0.3	3.2	"	(7.1)	(8.0)	---	2.9	4.2	---	---
5.6	17.3	0.6	3.6	"	(7.7)	(8.7)	---	6.3	10.7	---	---
5.4	19.7	0.5	3.6	"	(8.7)	9.7	---	7.9	13.5	---	5.9
5.1	19.0	0.4	---	"	---	---	---	---	---	---	---
5.0	12.5	0.7	3.5	"	(8.3)	9.3	---	6.3	10.7	---	5.6
4.8	12.5	0.5	3.5	"	(9.2)	(10.3)	---	7.3	12.0	---	5.9

[a] The value in parenthesis were converted from measurements at 18-21°C (the RTIC unit fluctuated during these runs).

low (of the order of 10^{-5} moles of nucleotides/liter), it is unnecessary to study the concentration dependence of the apparent molecular weight. The speeds for these runs were 3,189, 3,379 and 3,848 rpm for neutral solutions and 2,695 rpm for acidic ones. The partial specific volume, $\bar{v}$, was assumed unchanged with pH and a value of 0.546 (Eisenberg & Felsenfeld, 1967) was used. The "low-speed" method [Eq. (18)] was applied for calculating the weight-average molecular weight of poly(A) (Table III). The molecular weight becomes double at pH below 5.6 in accord with the model of a double-stranded form in acidic solution.

ii. β-Function and $\Phi^{1/3}P^{-1}$-Function

The molecular weight of poly(A) can be calculated by combining the sedimentation coefficient (s) and the diffusion coefficient (D) according to the Svedberg equation [Eq. (14)]. It can also be estimated by combining the s and [η] according to Eq. (28) either as a prolate ellipsoid of revolution (β-function) or as a flexible coil ($\Phi^{1/3}P^{-1}$-function).

Following the Oncley (1941) treatment and assuming no hydration, we have

$$[\eta] = \nu\bar{v}/100 \qquad (29)$$

Thus, with known [η] and $\bar{v}$, ν can be calculated. The axial ratio, p, and thereby the β-value can be estimated (Yang, 1961). If hydration is taken into account by replacing ν in Eq. (29) with ν_h $(1 + w/\bar{v}\rho)$, where w is grams of water of hydration per gram of polymer. The axial ratio and the β-value so determined will be slightly smaller than those without hydration. From Eq. (14) and Eq. (28), we have

$$M_\beta^{2/3} \equiv N s^\circ [\eta]^{1/3} \eta_\circ / \beta(1-\bar{v}\rho) \qquad (30)$$

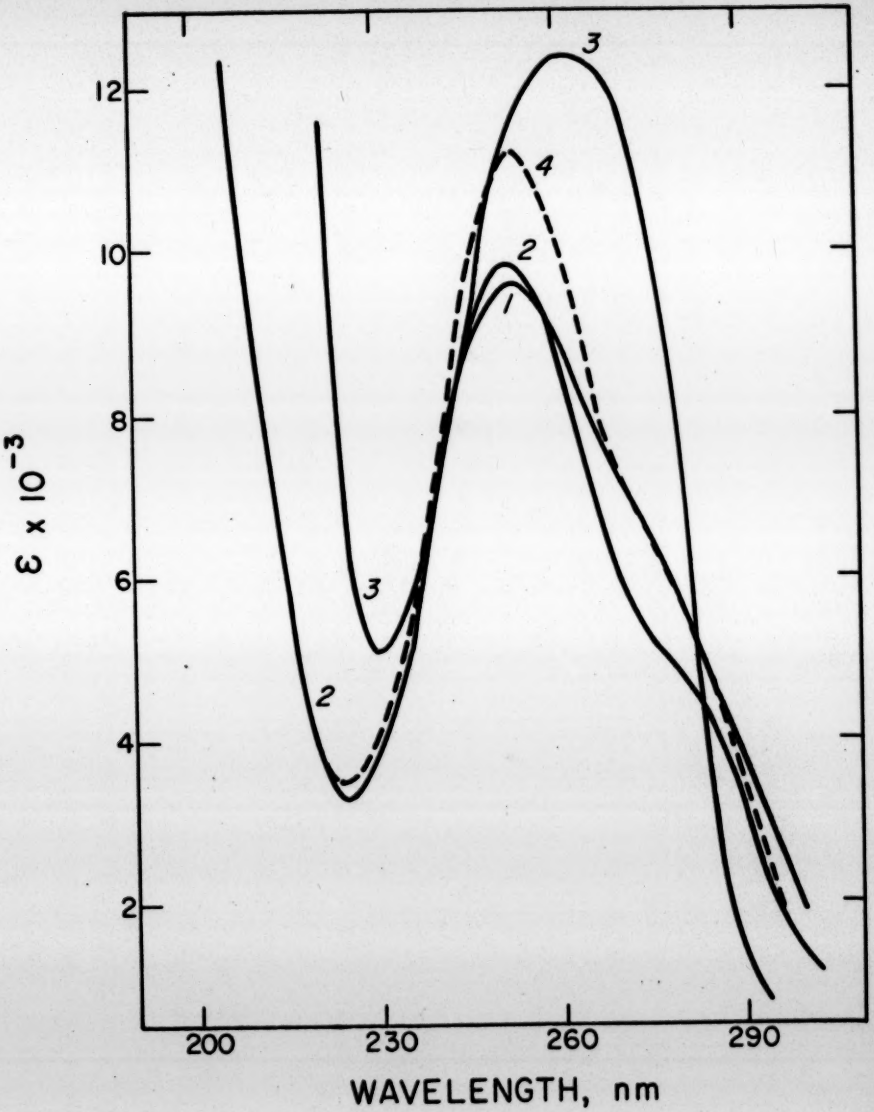
Therefore M_β will be somewhat larger when hydration is taken into consideration. For example, if w = 0.3 and $\bar{v}$ = 0.546 for poly(A), the ratio of ν/ν_h is equal to 1.5 and thusly p/p_h = 1.2-1.3. This will only result in about 2% difference in the β-value and 3% in M_β. Since the M_β is usually expected to be accurate to ± 10%, therefore we did not consider the factor of hydration in our calculations. Table III lists the M_β and $M_{\phi^{1/3}p^{-1}}$ of poly(A) at various pHs along with molecular weights determined by other methods. It is obvious from Table III that M_β agrees better with $M_{sed.\ eq.}$ than $M_{\phi^{1/3}p^{-1}}$. Thus, a model of prolate ellipsoid of revolution seems to fit the poly(A) molecule better than a flexible coil.

B. Polyriboguanylic Acid

1. Optical Studies

Figure 8 shows the UV absorption spectra of poly(G) in 0.01 M KF at pH 2.2, 7, 12.3 and 7 back titrated from pH 12.3. The spectrum at pH 2.2 (curve 1) is similar to that at pH 7 (curve 2) except that the absorption maximum and the shoulder near 280 nm show a slight red-shift in acidic solution. In addition the spectrum at pH 2.2 is slightly hyperchromic in the range of 300-260 nm and hypochromic at the absorption maximum relative to that at pH 7. In contrast, the spectrum is red-shifted by about 10 nm when the pH of the solution is raised to 12.3 and shows marked hyperchromicity near the absorption maximum. Furthermore, the shoulder near 280 nm disappears and the absorption band becomes broader than that at neutral pH. The spectrum at pH 12.3 (curve 3) resembles closely that of GMP in alkaline solution

Figure 8: UV absorption spectra of poly(G) in 0.01 M KF at different pHs. pH: 1, 2.2; 2, 7; 3, 12.3; 4, 7 back titrated from pH 12.3.


12
10
8
6
4
2
ε x 10⁻³
200
230
260
290
WAVELENGTH, nm
1
2
3
4

(Fresco & Massoulie', 1963). Pochon and Michelson (1965) had reported similar spectra of poly(G) in water at pH 1.18, 6, and 12.15, but their absorptivity at pH 12.15 was very much smaller. Back titrating the solution after one hour at pH 12.3 to pH 7 does not completely reverse the spectrum (curve 4).

Spectrophotometric titration of poly(G) in 0.1 M NaCl at 260 nm shows a sharp increase in absorbance between pH 11 and 12 with the midpoint at pH 11.4 (Fig. 9). Pochon and Michelson (1965) also reported a pK_a of 11.43 for poly(G) in 0.15 M NaCl as determined spectrophotometrically. The two curves in figure 9 represented the results on two batches of the polynucleotide.

Figure 10 shows the ORD spectra of poly(G) (Lot No. M: 11-08-314) in 0.1 M NaCl at five pHs. All measurements were made after the pH of the solution was adjusted for at least an hour. The general feature of the spectrum at pH 6.5 above 220 nm agrees with those previously reported (Arya, 1968; Green & Mahler, 1970) except slight difference in both the magnitude and the positions of the extrema. Between pH 6.5 and 10, no detectable changes in the ORD spectrum can be observed. Above pH 10 both the magnitudes of the Cotton effects and the positions of the extrema begin to change. The changes become even more drastic at pH 11.5, where the rotations are practically zero over the range of 240-300 nm. With pH above 11.6, the ORD spectrum shows only a small negative Cotton effect in agreement with that at pH 12.2 in 0.15 M salt by Ulbricht et al. (1966). The corresponding CD spectrum of poly(G) in 0.1 M NaCl (Fig. 11) displays the same alkaline pH-induced changes. Figure 12 shows the Cotton effects of poly(G) below 230 nm. In order to extend the measurements to 185 nm, 0.01 M KF was used as solvent. At pH 7 there is a negative extremum at 208 nm. At pH 12.2, the measurements

Figure 9: Alkaline titration of poly(G) in 0.1 M NaCl at 260 nm. ○ , Lot No. M: 11-08-314; ● , Lot No. M: 11-06-314.

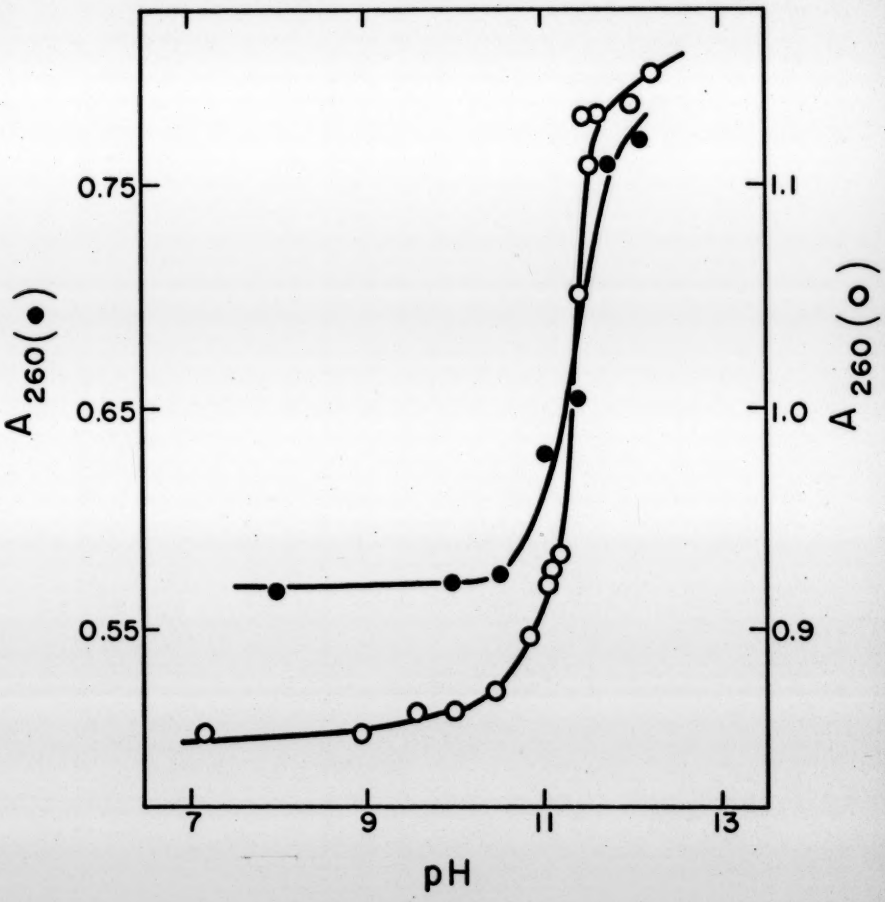

0.75
0.65
0.55
A260(●)
1.1
1.0
0.9
A260(○)
7
9
11
13
pH

Figure 10: ORD spectra of poly(G) in 0.1 M NaCl at different pHs. (Lot No. M: 11-08-314)

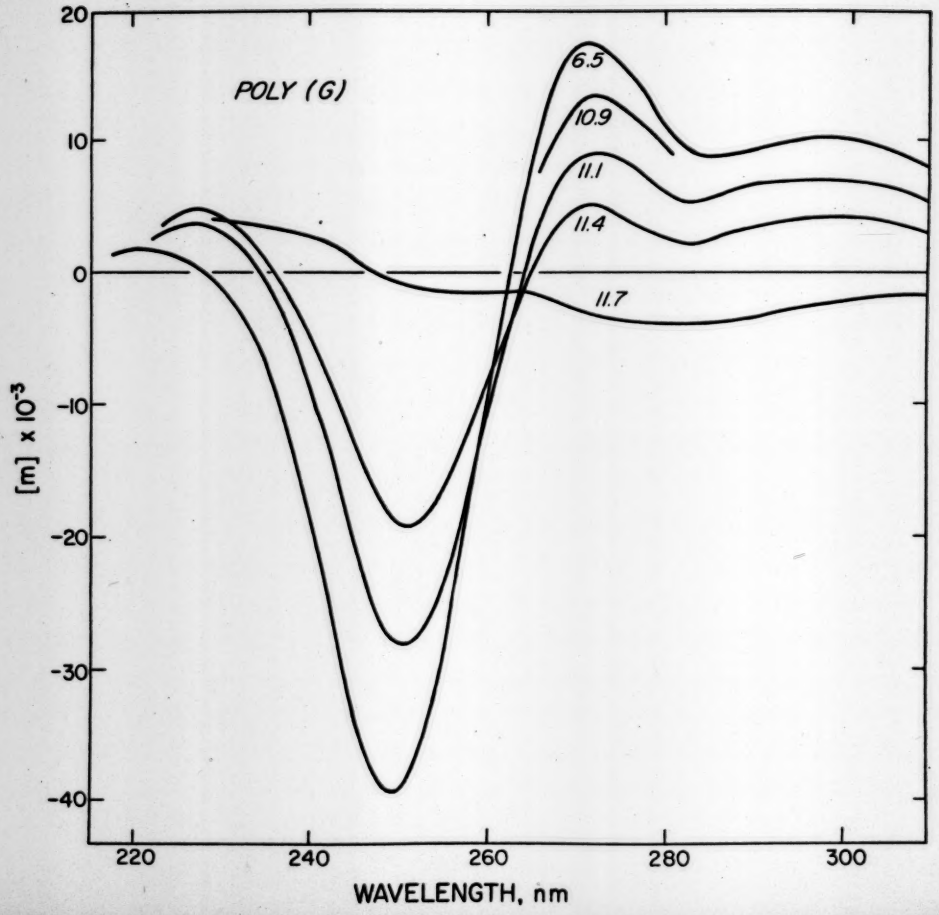

POLY (G)
6.5
10.9
11.1
11.4
11.7
20
10
0
-10
-20
-30
-40
[m] x 10⁻³
220
240
260
280
300
WAVELENGTH, nm

Figure 11: CD spectra of poly(G) in 0.1 M NaCl at different pHs. (Lot No. M: 11-08-314)

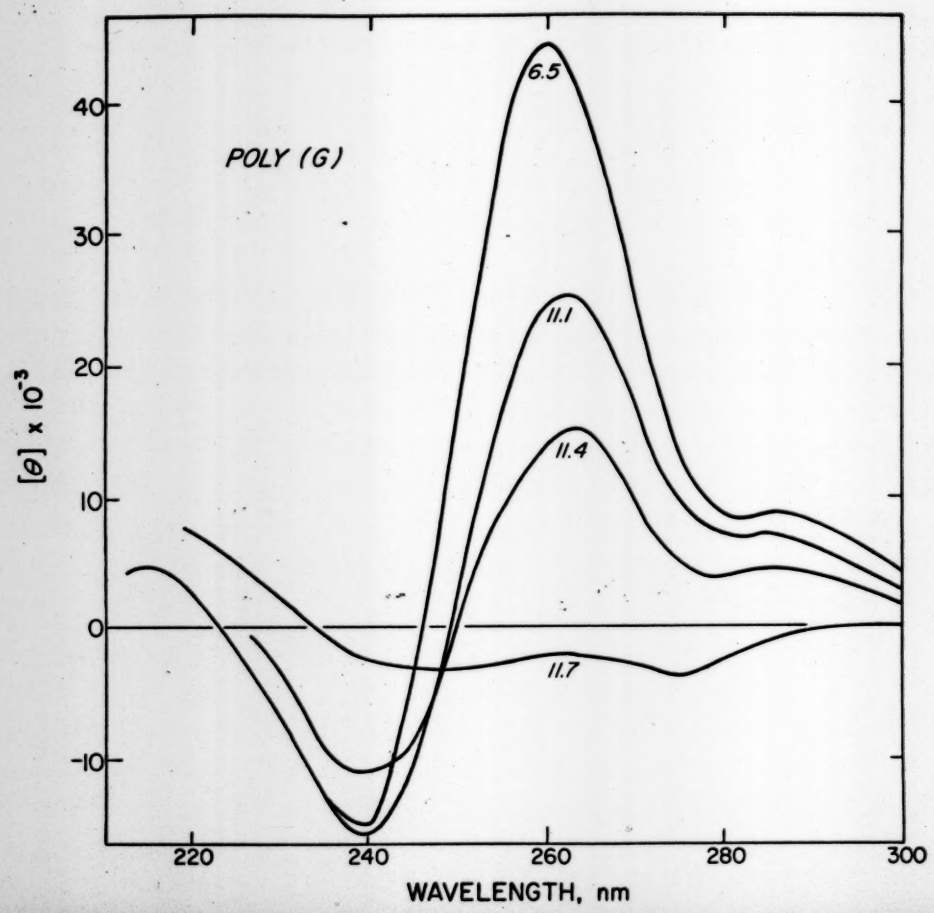
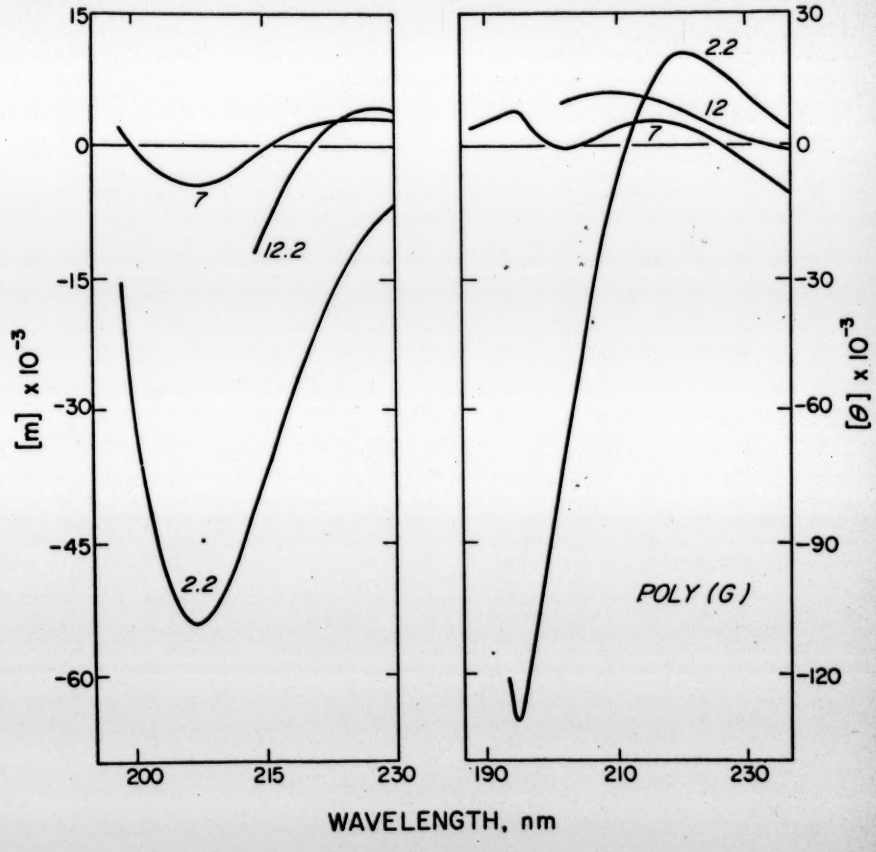

POLY (G)
6.5
11.1
11.4
11.7
40
30
20
10
0
-10
[θ] x 10⁻³
220
240
260
280
300
WAVELENGTH, nm

Figure 12: ORD and CD spectra of poly(G) in 0.01 M KF at pH 2.2, 7 and 12 below 230 nm.


15
0
-15
-30
-45
-60
[m] x 10^-3
7
12.2
2.2
200
215
230
30
0
-30
-60
-90
-120
[θ] x 10^-3
2.2
12
7
POLY (G)
190
210
230
WAVELENGTH, nm

were limited to above 214 nm, but the levorotations seemed to increase at lower wavelengths. The corresponding CD spectrum at pH 7 shows two small positive extrema at 215 and 195 nm and one small negative extrema at 203 nm, which are in qualitative agreement with the observation in 0.1 M potassium phosphate at pH 7 by Wolfe et al. (1969). But their corresponding extrema were located at 215, 190 and 205 nm and the shape of the bands was more symmetrical. Also included in Fig. 12 is the ORD & CD of poly(G) at pH 2.2 which are markedly different from those at neutral and alkaline pH.

The variations in the magnitude of the ORD and CD extrema with pH (Fig. 13) indicate a drastic conformational change for poly(G) between pH 11 and 12. Furthermore, this conformational change is salt dependent. For example, the midpoint of the transition is shifted from pH 11.4 in 0.1 M NaCl to 11.75 in 0.01 M KF in accord with the results of spectrophotometric titration (Pochon & Michelson, 1965). The rotations of poly(G) in alkaline solution change with time; even the sign of the rotations at the extrema is reversed (Table IV). Comparing the spectrum in 0.1 M NaCl at pH 11.7 (Figure 10) and the data in 0.01 M KF at pH 11.7 (Table IV), we observed the effect of salt concentration on the time to reverse the sign of the rotations. The multiple Cotton effects at pH 7 could be recovered by back titration from pH 12, provided the solution was kept at pH 12 for less than 30 minutes at room temperature or less than three hours at 4°C (Table IV).

Figure 14 shows the elution patterns of poly(G) in 0.01 M KF at pH 7 on a Sephadex G-200 column eluted with the same solvent. The sample at neutral pH is eluted out with the marker, blue dextran 2,000, and displays only a single peak at fraction No. 50 (point a). The sample treated with 7 M urea shows the same pattern. After one-hour exposure

Figure 13: Variations of the ORD and CD extrema with alkaline pH at 25°C.

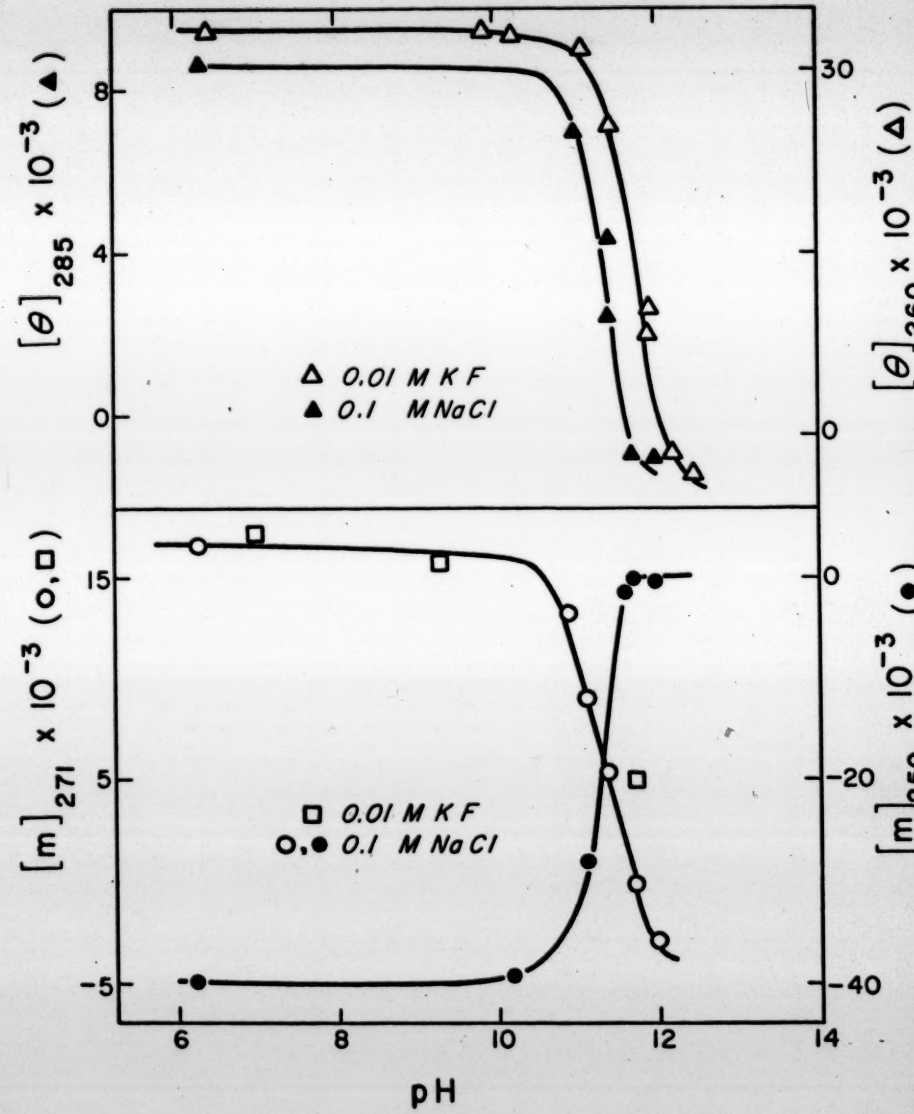

$[\theta]_{285} \times 10^{-3}$ (▲)
$[\theta]_{260} \times 10^{-3}$ (△)
△ 0.01 M KF
▲ 0.1 M NaCl
$[m]_{271} \times 10^{-3}$ (○,□)
$[m]_{250} \times 10^{-3}$ (●)
□ 0.01 M KF
○,● 0.1 M NaCl
pH

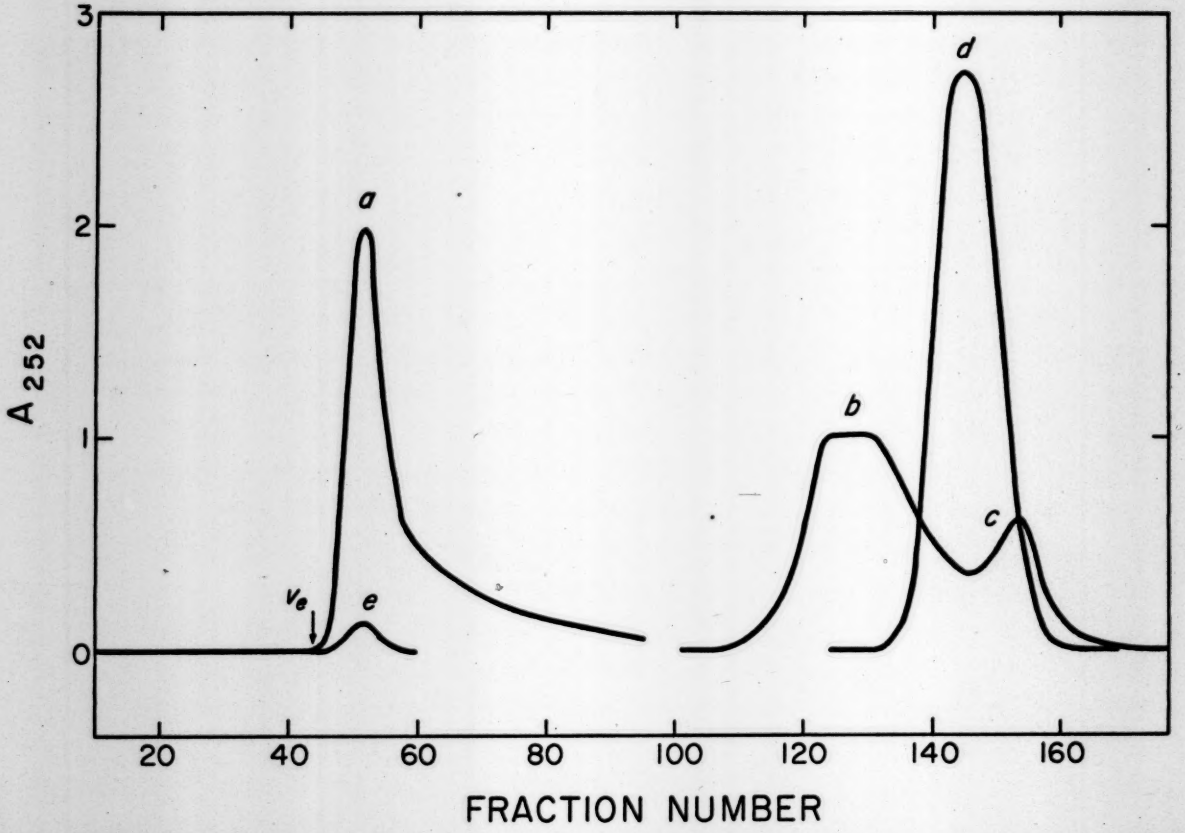
Table IV

Time Dependence of the Optical Rotation of Poly(G) in Alkaline Solution[a]

Solvent (0.01 M KF)	$[m]_{271} \times 10^{-3}$	$[m]_{249} \times 10^{-3}$
1. pH 7, r.t.[b]	19.3	-32.0
2. pH 10.9, 24 hr., r.t.	10.7	-17.0
3. pH 11.7, 24 hr., r.t.	5.3	-7.8
4. pH 12.3-12.5		
< 1 hr., r.t.	0.3-0.5	1.3-1.5
6 hr., r.t.	0.0	1.5
12 hr., r.t.	-1.0- -3.0	1.8-2.0
24 hr., 4°C	0.0- -0.6	1.3-1.8
(back titration to pH 7 from alkaline solution)		
1. pH 12.3, r.t.		
< 30 min.	19.0	-30.8
2 hr.	14.3	-21.8
3 hr.	11.2	-17.3
6 hr.	7.6	-10.7
16 hr.	1.0	-2.7
2. pH 12.4, 4°C		
3 hr.	18.8	-28.0
15 hr.	12.2	-16.6

[a] All measurements were made at 25°C.
[b] Abbreviation for room temperature

Figure 14: Elution pattern of poly(G) in 0.01 M KF at room temperature on a Sephadex G-200 column using 0.01 M KF at pH 7 as the eluent. Curves: a, pH 7; b & c, titrated to pH 12.4 for 1 hr; d, pH 12.4 for 24 hr; e, blue dextran; Ve, void volume (0.6 ml/fraction).


3
2
1
0
A_{252}
a
b
c
d
e
V_e
20
40
60
80
100
120
140
160
FRACTION NUMBER

at pH 12.4, the polynucleotide comes out much later and shows a large peak (about three fourth of the sample) at fraction No. 125 (point b), followed by a small peak at fraction No. 155 (point c). After 24 hours' standing at pH 12.4, the sample shows only one peak at fraction No. 145 (point d). Similar results are obtained for poly(G) in 0.05 M cacodylate buffer containing 0.1 M $NaClO_4$ on Agarose A-15 m column. The CD of the three pooled fractions is shown in Fig. 15 where the symbols a, b and c refer to the fractions shown in Fig. 14. Curve a is identical with that of the sample before passing through the column. Curve b resembles curve a, but the small 290-nm shoulder almost disappears; also the peak and the trough are red-shifted and slightly intensified relative to that of curve a. The Cotton effects of curve c are greatly diminished. The diminution is even more pronounced with the pooled fraction d (not shown) for which the ellipticity is practically zero over the range of 300-260 nm. The CD of curve d (not shown) is similar to that of purine β-mononucleotides, thus suggesting a complete loss of the secondary structure of poly(G). Although the number of strands of poly(G) at neutral pH is not known, the data of ORD, CD and UV absorbance as well as the gel chromatography in alkaline solution suggest the dissociation of strands of poly(G). Further evidence of dissociation in alkaline solution comes from the study of sucrose-gradient sedimentation. In 0.01 M KF at pH 7, the polynucleotide is located between the markers of 23 S and 16 S ribosomal RNAs, but the alkaline-treated sample stays on top of the 16 S markers.

Figure 16 and 17 show the ORD and CD of poly(G) (Lot No. M: 11-06-314) in solvent B (0.01 M sodium citrate-HCl buffer) at pH 7, 3.1, 2.8 and 2.5. We use low salt because the acidic pK_a value is higher at lower ionic strength (see Section IB), thus enabling us to

Figure 15: CD spectra of poly(G) in 0.01 M KF eluted from a Sephadex G-200 column. a, b, c, denoted the pooled fractions in Figure 14.

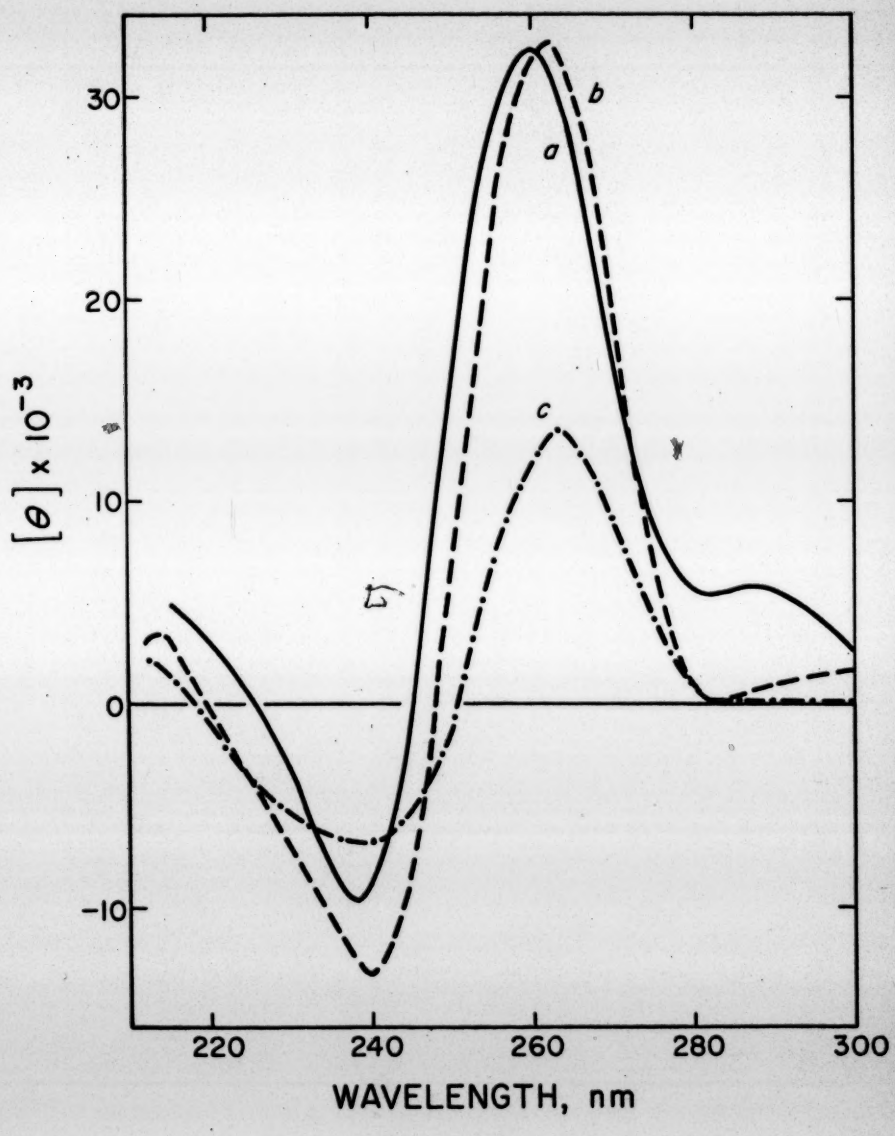

30
20
10
0
-10
[θ] x 10^-3
a
b
c
220
240
260
280
300
WAVELENGTH, nm

Figure 16: ORD spectra of poly(G) in 0.01 M sodium citrate-HCl buffer at pH 7, 3.1, 2.8 and 2.5. (Lot No. M: 11-06-314)

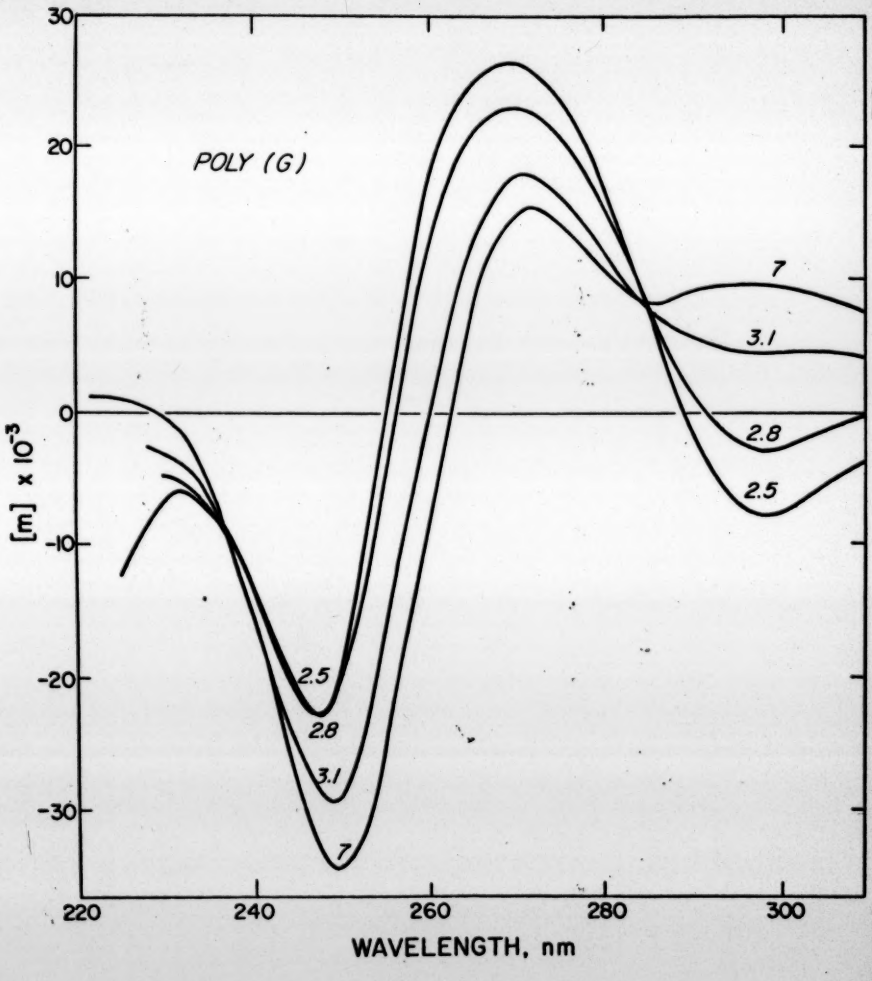
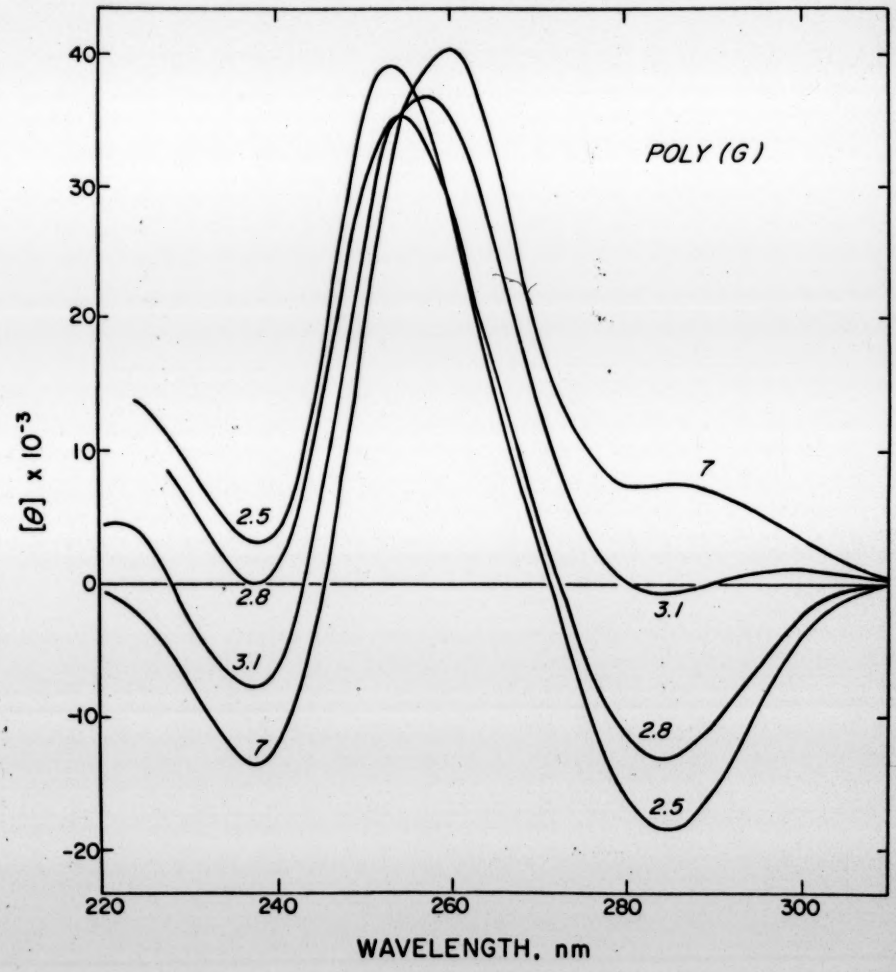

POLY (G)
7
3.1
2.8
2.5
2.5
2.8
3.1
7
30
20
10
0
-10
-20
-30
220
240
260
280
300
[m] x 10⁻³
WAVELENGTH, nm

Figure 17: CD spectra of poly(G) in 0.01 M sodium citrate-HCl buffer at pH 7, 3.1, 2.8 and 2.5. (Lot No. M: 11-06-314)


POLY (G)
40
30
20
10
0
-10
-20
[θ] x 10⁻³
220
240
260
280
300
WAVELENGTH, nm
2.5
2.8
3.1
7
7
3.1
2.8
2.5

follow the conformational changes in a convenient pH range. Between pH 7 and 3.5, no significant changes are observed for both ORD and CD. Below pH 3.5, both ORD and CD show a decrease in magnitude of the long-wavelength shoulder and the extrema around 240 nm, but an increase in the ORD peak near 270 nm. Also the extrema around 260-270 nm of both ORD and CD are blue-shifted. Our results differ from the report by Ulbricht et al. (1966) who found that in 0.15 M salt the rotation of poly(G) changed little as the pH was reduced to 1 and the positions of the extrema were red-shifted. Below pH 3.1, our results agree qualitatively with those reported in the literature, namely the positive extrema of ORD at 298-300 nm and CD at 282-285 nm in neutral pH become negative (Cohen et al., 1969; Wolfe et al., 1969; Thiele & Guschlbauer, 1971) even though under different experimental conditions. Unlike in alkaline solutions, these acid-induced changes in optical properties are reversible, when the poly(G) in acidic solution is dialyzed back to pH 7. Below 230 nm, we find another large trough at 207 nm for ORD of poly(G) in 0.01 M KF at pH 2.2 (see Figure 12). Similarly, there are two CD bands below 230 nm in accord with that in water at pH 3.3 observed by Wolfe et al. (1969) but their extrema were located at wavelengths 2-3 nm longer than ours.

As in alkaline solution, poly(G) (Lot No. M: 11-06-314) in solvent B undergoes a sharp conformational change between pH 2.5 and 3.5 with a midpoint at pH 3 (Fig. 18). Thiele and Guschlbauer (1971) had reported a pK_a of 2.75 in 0.01 M NaCl for poly(G) from Biopolymers Inc.

Tables V and VI list the pertinent data of ORD and CD of poly(G) between pH 2.2 and 11.7. In alkaline solution, the positions of the extrema are red-shifted with increasing pH, and in acidic solution they are blud-shifted with decreasing pH. The extremum 4 is the only

Figure 18: Changes of the ORD and CD extrema of poly(G) with pH in 0.01 M sodium citrate-HCl. (Lot No. M: 11-06-314)

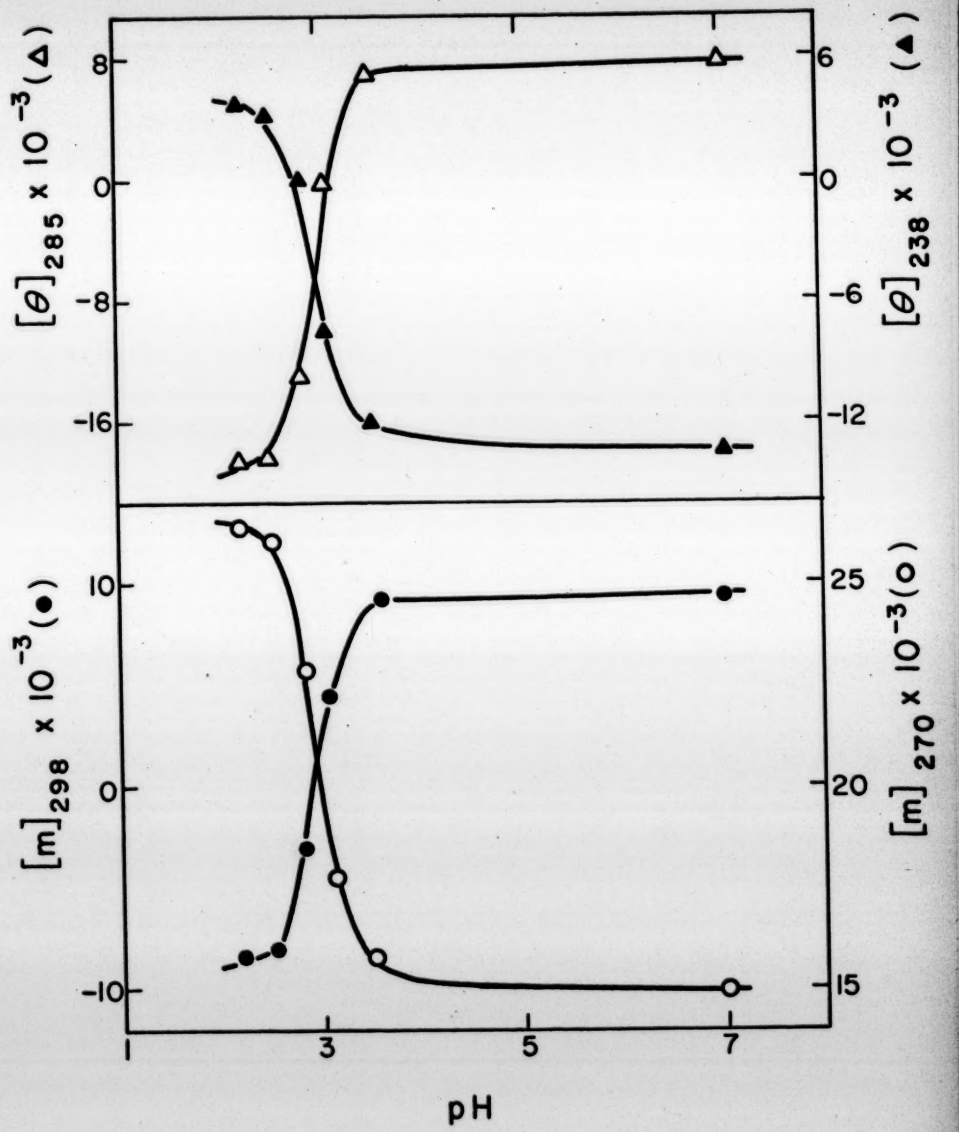

[θ]285 x 10-3(Δ)
[θ]238 x 10-3 (▲)
[m]298 x 10-3(●)
[m]270 x 10-3(○)
8
0
-8
-16
6
0
-6
-12
10
0
-10
25
20
15
1
3
5
7
pH

Table V

The Optical Rotatory Dispersion of Poly(G) at 25°C[a]

Solvent pH	Extremum 1 λ	Extremum 1 [m]	Extremum 2 λ	Extremum 2 [m]	Extremum 3 λ	Extremum 3 [m]	Extremum 4 λ	Extremum 4 [m]
(in 0.01 M KF[b])								
7.0	293	10.3	271	19.0	249	-32.0	222	3.5
(in 0.1 M NaCl)								
6.5	297	10.1	271	17.3	249	-39.0	221	1.8
10.9	"	9.3	272	13.3	---	---	---	---
11.1	"	7.2	"	9.1	251	-28.2	227	3.6
11.4	298-301	4.1	"	5.3	"	-19.3	"	5.0
> 11.7	---	---	283	-4.0	---	---	228	3.6
(in 0.01 M Sodium Citrate-HCl Buffer)								
3.9	299-301	9.2	271	14.7	250	-33.8	221	1.1
3.1	298-300	4.3	"	17.8	248	-29.5	---	---
2.8	298	-3.0	269-270	22.8	247	-23.3	---	---
2.2	298-299	-8.1	270	26.4	"	-23.2	231	-6.0

[a] [m] in 10^3 deg cm^2/decimole and λ in nm

[b] Other extrema in 0.01 M KF: at pH 7.0, $[m]_{208} = -4.5$ and at pH 2.2, $[m]_{207} = -54.0$

Table VI

The Circular Dichroism of Poly(G) at 25°C[a]

Solvent	Extremum 1		Extremum 2		Extremum 3		Extremum 4	
pH	λ	[θ]	λ	[θ]	λ	[θ]	λ	[θ]
(in 0.01 M KF[b])								
7.0	288	6.4	260	38.1	239	-11.5	214	5.2
(in 0.1 M NaCl)								
6.5	285	8.7	260	46.1	239	-15.2	215	4.8
11.1	"	7.0	263	25.3	"	-15.8	---	---
11.4	"	4.0	"	15.1	240	-10.8	---	---
> 11.7	---	---	274-5	-4.3	248	-3.5	208	12.0
(in 0.01 M Sodium Citrate-HCl Buffer)								
7.2	285	7.5	260	40.4	237-8	-13.5	---	---
3.5	281-5	7.0	258	40.2	237-9	-12.1	---	---
3.1	298-9	1.1	---	---	---	---	---	---
	282-5	-0.2	"	36.9	237	-7.8	222	4.4
2.8	283-5	-13.0	254	35.5	237-8	0	---	---
2.2	284-5	-18.5	253-4	39.2	238	3.6	222	18.0

[a] $[\theta]$ in 10^3 deg cm^2/decimole and λ in nm

[b] Other extrema in 0.01 M KF: at pH 7.0, $[\theta]_{203} = -0.5$; $[\theta]_{195} = 7.6$ and at pH 2.2, $[\theta]_{195} = -131$

exception; its ORD position is shifted from 221 nm at pH 7 to 231 nm at pH 2.2 and its CD position from 215 nm at pH 7 to 222 at pH 2.2 and 208 nm at pH 11.7.

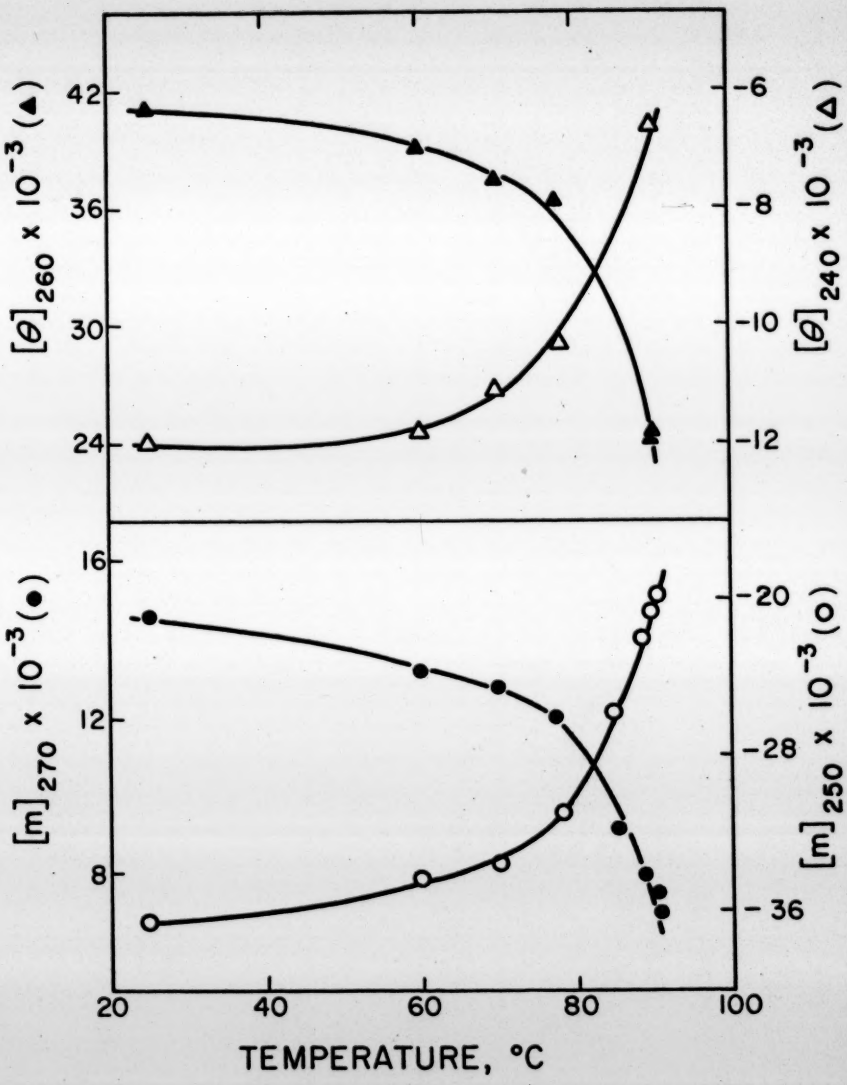
At pH 2.0 in solvent B, the ORD and CD of poly(G) show no marked changes between 25° and 70°C. The magnitude of the Cotton effects is slightly decreased at high temperatures. At 90°C the decrease in ORD is about 15% for $[m]_{246}$ and 20% for $[m]_{270}$. There is no indication of precipitation of the polymer even at temperature higher than 90°C. On the other hand, in the absence of added salt poly(G) shows aggregation at pH 3.3 and at room temperature. When this solution is passed through the Millipore filter type SC (8 μ), the filtrate showed very little decrease of absorbance. With type HA (0.45 μ) filter, however, it decreases by about 50%. The filtrate shows an ORD spectrum that is qualitatively similar to that in solvent B at pH below 2.8 and 25°C. Further precipitation of poly(G) without salt occurs upon heating the solution to 45°C.

At neutral pH without added salt, poly(G) shows a temperature-induced transition (Fig. 19). The melting curve is not complete even at 92°C and there is no indication of any precipitation even at this high temperature.

2. Hydrodynamic Studies

The intrinsic viscosity of poly(G) (Lot No. M: 11-08-314) in solvent C (0.1 M NaCl plus 0.05 M cacodylate buffer) at 25°C is 0.33 dl/gm at pH 7.5, which drops to 0.11 dl/gm at pH 11.3 in 0.15 M Na_2HPO_4. (The solutions were prepared by dissolving the sample in 0.1 M NaCl, pH 7 and then dialyzing against the solvent for at least 12 hours at 4°C before measurements). In solvent B, poly(G) (Lot No. M: 11-06-314)

Figure 19: Variations of the CD and ORD extrema of poly(G) in H_2O (pH 6.5) with temperature.


42
36
30
24
$[\theta]_{260} \times 10^{-3}$ (▲)
-6
-8
-10
-12
$[\theta]_{240} \times 10^{-3}$ (△)
16
12
8
$[m]_{270} \times 10^{-3}$ (●)
-20
-28
-36
$[m]_{250} \times 10^{-3}$ (○)
20
40
60
80
100
TEMPERATURE, °C

at pH 7 has an [η] equal to 0.29 dl/gm. The reduced viscosity for a 0.04% solution is 0.34 dl/gm at pH 7 and 0.30 dl/gm at pH 3.1.

The sedimentation (s) and diffusion (D) coefficients of poly(G) (Lot No. M: 11-08-314) in solvent C at pH 7 were determined with the schlieren optics at 25°C. In another experiment the s value was measured at extremely low concentration (10^{-4} M) with the photoelectric scanner. It agreed exactly with the extrapolated s° done with the schlieren optics.

The s° of poly(G) (Lot No. M: 11-08-314) in 0.15 M salt is constant between pH 7 and 10, above which it drops markedly with increasing pH (Table VII). Furthermore, the s° becomes time- and temperature-dependent; for example, the s°_{20} of poly(G) in 0.01 M KF changes from 9.8 to 4.0 S after 24 hours at pH 12.2 at 4°C and to 2.2 S if the solution is kept at room temperature for about 12 hours. In solvent B, the s° of poly(G) (Lot No. M: 11-06-314) increases gradually upon lowering the pH of the solution. The change in s°_{20} is small (from 7.2 to 7.3 S) between pH 7 and 3.5. Within this range there is also no significant changes in optical properties. As the pH is further lowered, the s°_{20} continues to increase (see Table VII) and the solution shows aggregation below pH 2.2 as judged by the decrease of its absorbance upon passing through the Millipore filters.

3. Determination of Molecular Weight

By combining the s°_{25} with D°_{25} or $[\eta]_{25}$, we estimated the molecular weight of poly(G) according to Equation (14) or (30) with $\bar{v} = 0.55$ (Tennent & Vilbrandt, 1943). The limited results in Table VIII make it difficult to decide whether a prolate ellipsoid of revolution or a flexible coil is a better model for poly(G). But it does show a five-

Table VII

Hydrodynamic Properties of Poly(G)

Solvent	$[\eta]_{25}$	s°_{20}	s°_{25}	D°_{25}
pH	dl/gm	S	S	x 10^{7}, cm^{2}/sec
M: 11-08-314 (in 0.1 M NaCl plus 0.05 M Cacodylate Buffer)				
7.5	0.33	8.5	9.5	2.6
M: 11-08-314 (in 0.15 M Na_2 H PO_4)				
11.3	0.11	4.0	4.5	---
11.6	---	1.2	1.3	---
M: 11-06-314 (in 0.01 M Sodium Citrate-H Cl Buffer)				
7.0	0.29	7.2	8.1	---
3.5	---	7.3	8.2	---
2.8	---	8.0	9.0	---
2.5	---	8.2	9.2	---

Table VIII

Molecular Weight Determination of Poly(G)

Method	pH 7.5	pH 11.3
M_{s-D}	2.0×10^5	---
M_{β}[a]	1.6×10^5	0.32×10^5
$M_{\Phi^{1/3}P^{-1}}$	1.8×10^5	0.32×10^5

[a] Assuming $\beta = 2.7 \times 10^6$ for pH 7.5 and 2.5×10^6 for pH 11.3

to six-fold decrease in molecular weight when the pH of the solution is raised from 7.5 to 11.3, indicating a dissociation of the strands of poly(G).

C. Polyribocytidylic Acid

1. Optical Studies

Figure 20 shows the UV absorption spectra of poly(C) in solvent A (0.08 M NaCl and 0.02 M sodium citrate plus citric acid). Lowering the pH from 6.2 to 4.8 results in an abrupt red-shift and hyperchromic effect of the absorption maximum. Ts'o et al. (1962) has reported that CMP also shows similar changes upon protonation. At pH 3.6, the absorbance is further increased but the absorption maximum is only slightly red-shifted. These changes in the absorption spectra of poly(C) are attributed to the protonation of the bases (Ts'o et al., 1962) with a pK_a of 5.7 in 0.1 M NaCl, as determined by Hartman and Rich (1965).

Figures 21 and 22 show the ORD and CD of poly(C) in solvent A at various pHs. The solid lines represent the spectra at pH 6.2, where poly(C) is single-stranded with bases partially stacked (Michelson et al., 1967; Gulik et al., 1970), and at pH 4.95 where it is believed to be a parallel double-stranded helix (Akinrimisi et al., 1963; Langridge & Rich, 1963). However, our results indicate that poly(C) in acidic solution adopts a hairpin structure rather than a dimer (see Section III C2). The general features of the ORD and CD in figures 21 and 22 of these two different conformations agree very well with those in the literature (Fasman et al., 1964; Sarkar & Yang, 1965b; Ts'o et al., 1966; Adler et al., 1967, 1969; Guschlbauer, 1967; Brahms et al., 1967; Wolfe et al., 1969; Green & Mahler, 1970;

Figure 20: UV absorption spectra of poly(C) in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at pH 6.2, 4.8 and 3.6.

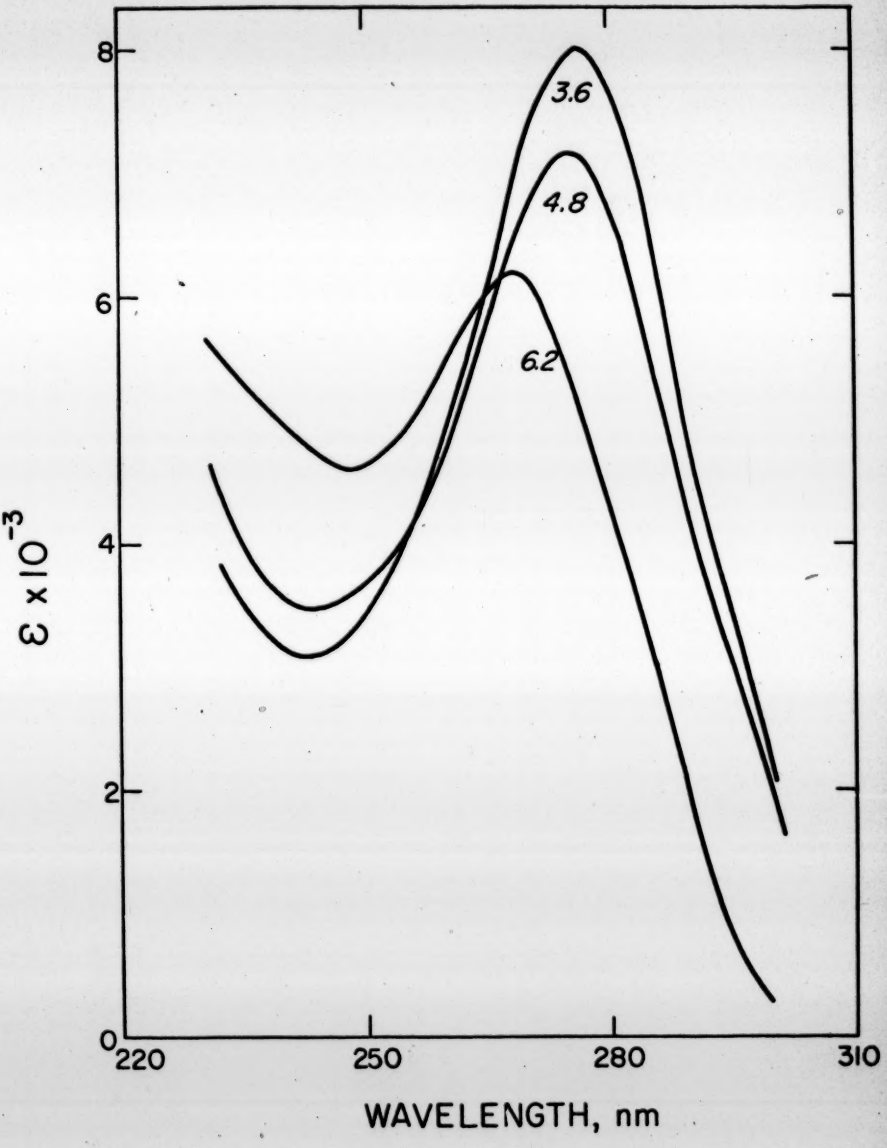

8
6
4
2
0
3.6
4.8
6.2
ε x10⁻³
220
250
280
310
WAVELENGTH, nm

Figure 21: ORD spectra of poly(C) at 25°C in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at different pHs. (Lot No. S: 6701)

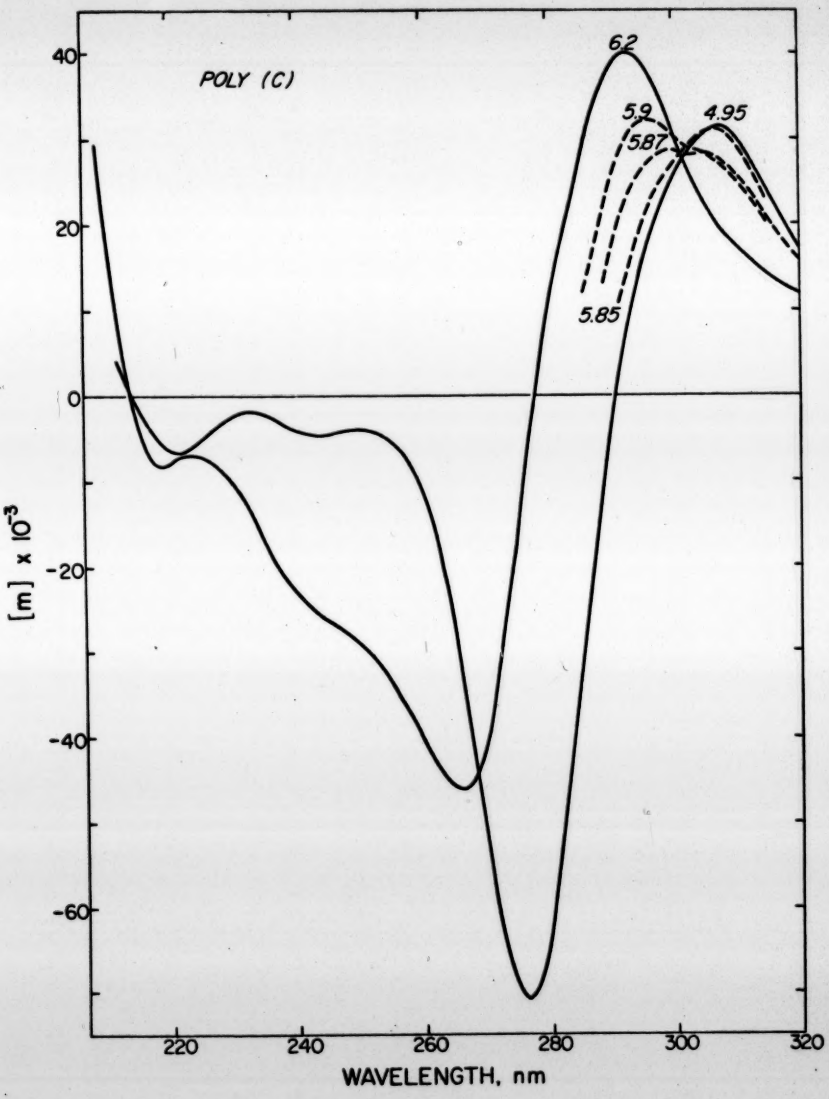
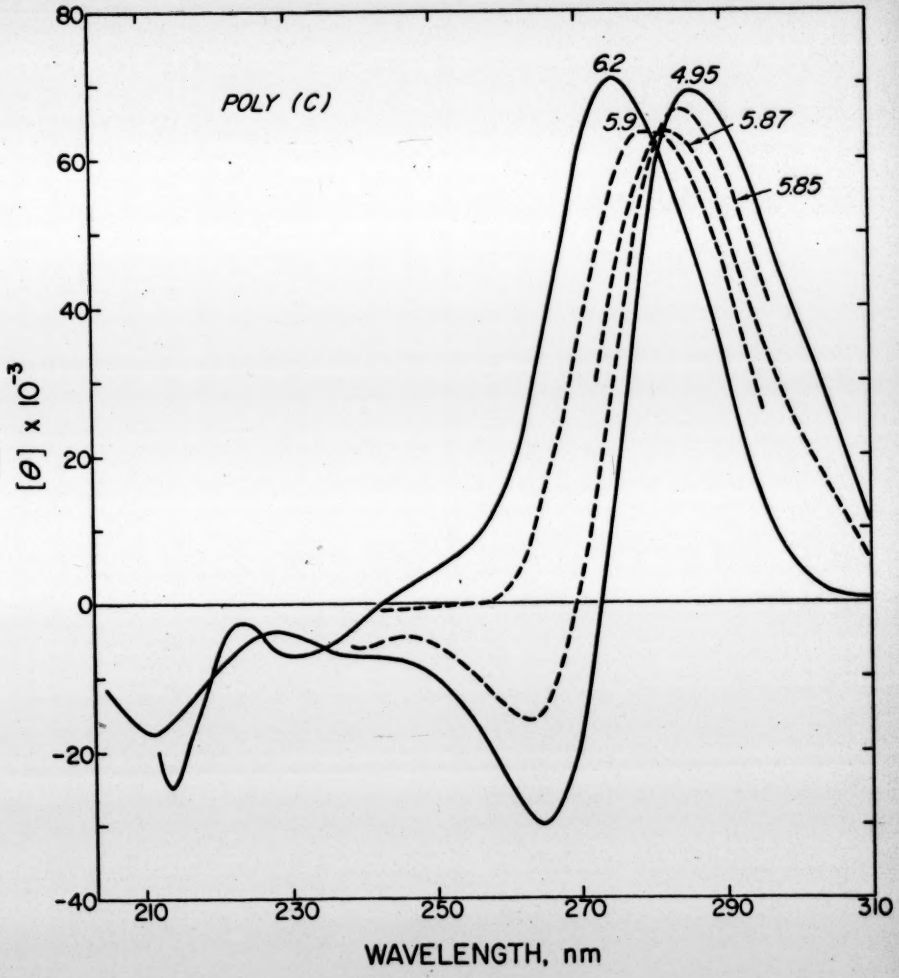

POLY (C)
6.2
5.9
5.87
5.85
4.95
40
20
0
-20
-40
-60
[m] x 10⁻³
220
240
260
280
300
320
WAVELENGTH, nm

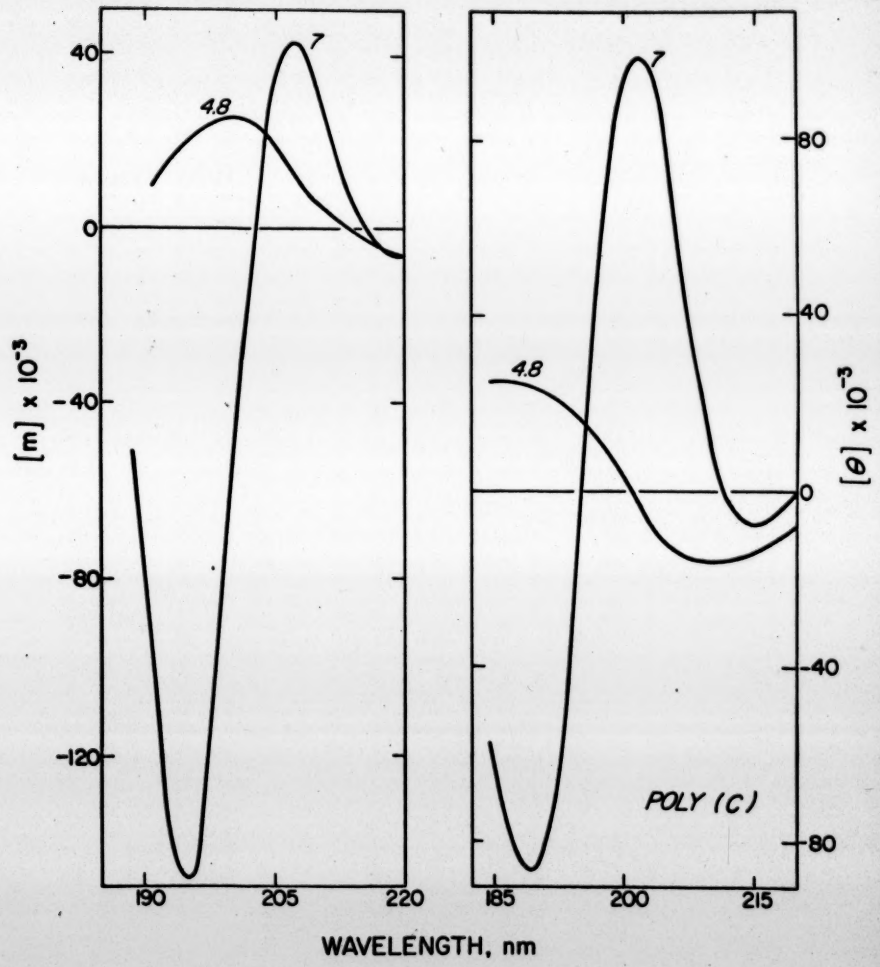
Figure 22: CD spectra of poly(C) at 25°C in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at different pHs. (Lot No. S: 6701)


POLY (C)
6.2
4.95
5.9
5.87
5.85
80
60
40
20
0
-20
-40
[θ] x 10^-3
210
230
250
270
290
310
WAVELENGTH, nm

Sarocchi et al., 1970; Gulik et al., 1970). On changing the pH from 6.2 to 4.95, the ORD is greatly red-shifted and the magnitude of the Cotton effects decreases, whereas the CD is changed from nonconservative towards more conservative with the extrema red-shifted. The broken lines represent the spectra in the pH range of transition from single- to double-stranded form. The extrema of both ORD and CD at pH 5.9 are broadened, their magnitude decreased, and their positions red-shifted relative to those at pH 6.2. Furthermore, in addition to the peak of ORD at 295 nm, a shoulder at 305-307 nm appears. At pH 5.87, the ORD has two peaks and is further red-shifted relative to that at pH 5.9; similarly, the CD is red-shifted. At pH 5.85, the ORD shows only one peak at 307 nm which resembles that at pH 4.95; a similar change can be seen in the CD spectrum. Since this transition is in such a narrow pH range that the "structure" at each pH is very unstable; for example, the spectrum at pH 5.85 could be shifted to that at pH 5.9, merely on standing or by shaking up the poly(C) solution. Guschlbauer (1967) has also reported the unstability of the structure near the pK_a value, but we have no evidence that the structure of poly(C) collapses, as was noted by Guschlbauer (1967) at pH slightly above the pK_a value. Adler et al. (1969) also reported that the ORD of poly(C) from Miles Laboratories, Inc. had two peaks of equal magnitude at pH 5.7 (the pK_a value) in 0.1 M Na^+ salt. Our samples from Schwarz BioResearch, Inc. show the two peaks at pH 5.87 (Fig. 21) and those from Miles Laboratories, Inc. at pH 5.75.

Figure 23 shows the ORD and CD of poly(C) in 0.01 M KF at pH 7 and 4.8 below 220 nm. At pH 7 both ORD and CD display large Cotton effects between 190 and 210 nm, whereas at pH 4.8, the shape and the magnitude of these Cotton effects at short wavelengths are greatly diminished.

Figure 23: ORD and CD spectra of poly(C) in 0.01 M KF at pH 7 and 4.8 below 220 nm. (Lot No. S: 6701)


40
0
-40
-80
-120
[m] x 10⁻³
7
4.8
190
205
220
80
40
0
-40
-80
[θ] x 10⁻³
7
4.8
POLY (C)
185
200
215
WAVELENGTH, nm

Wolfe *et al.* (1969) also observed the disappearance of the large symmetrical CD bands of poly(C) in 0.1 M phosphate salt at pH 3.95. These changes of the Cotton effects with pH are completely reversible upon neutralization.

Table IX lists the pertinent numerical values of the Cotton effects of poly(C) from both Schwarz BioResearch Inc. and Miles Laboratories Inc.

2. Hydrodynamic Studies

Table X lists the intrinsic viscosities of poly(C) at different pHs. The intrinsic viscosity at pH 6.2 is much higher than that at pH 4.8. The flow time for water at 25°C was about 1,000 seconds and the concentration of the solution at all pHs was so chosen that the difference in flow time between the solution and solvent was at least 40 seconds. The Huggins' constant was between 0.4 and 1.4 in the pH range studied. Figure 24 shows that both the hydrodynamic and optical properties of poly(C) undergo a sharp transition in acidic solution with a midpoint at pH 5.87, suggesting a drastic change in conformation as well as in shape. Adler *et al.* (1969) also reported such a transition based on an ORD study.

Sedimentation velocity experiments were carried out at 20 and 25°C with the photoelectric scanner at 52,640 rpm. The sedimentation coefficients of poly(C) in solvent A at 25°C are 5.46, 7.52 and 7.47 S for solutions at pH 6.2, 4.8 and 3.6, respectively, and it is 6.51 S for solution at pH 4.8 and 20°C. Akinrimisi *et al.* (1963) and Hartman & Rich (1965) also observed the increase in s value upon lowering the pH of the solution. The sharp drop in $[\eta]$ (Fig. 24) in acidic solution and the increase in s° values suggest that the poly(C) molecule is more

Table IX

Cotton Effects of Poly(C)[a]

(in 0.08 M NaCl and 0.02 M Sodium Citrate plus Citric Acid at 25°C)

Solvent	ORD Extrema[b]						CD Extrema[b]					
pH (Lot No.)	λ_1	$[m]_1$	λ_2	$[m]_2$	λ_3	$[m]_3$	λ_1	$[\theta]_1$	λ_2	$[\theta]_2$	λ_3	$[\theta]_3$
S: 6701												
6.2	292-3	40.0	266	-46.1	224	-7.0	275	70.9	231	-7.1	233	-2.8
5.9	296	32.4	---	---	---	---	280	63.7	---	---	---	---
5.87	307	27.8	299	28.6	---	---	282-3	63.7	---	---	---	---
5.85	"	30.9	---	---	---	---	285	66.7	262	-15.8	---	---
4.95	"	31.7	276	-71.0	222	-6.5	"	69.2	265	-30.1	211	-17.4
3.6	"	28.7	"	-66.0	---	---	286	64.7	264-5	-27.7	---	---
M: 11-04-30												
6.2	293	39.7	266	-45.7	---	---	275	71.4	232	-6.6	---	---
5.85	297	29.0	270	-44.6	---	---	280-1	59.8	247	-0.4	---	---
5.75	305-6	26.4	300	26.2	272-3	-46.9	283	57.2	259	-7.2	---	---
5.4	306	26.5	273	-51.2	---	---	"	58.4	261	-11.9	---	---

[a] [m] and [θ] in 10^3 deg cm^2/decimole and λ in nm

[b] Other extrema of Lot No. S: 6701 in 0.01 M KF: at pH 6.2, $[m]_{207} = 42.0$; $[m]_{195} = 148$ and $[\theta]_{215} = -7.0$; $[\theta]_{202} = 99.0$; $[\theta]_{189} = -86.0$ and at pH 4.95, $[m]_{200} = 25.0$ and $[\theta]_{210} = -16.0$; $[\theta]_{186-7} = 26.0$

Table X

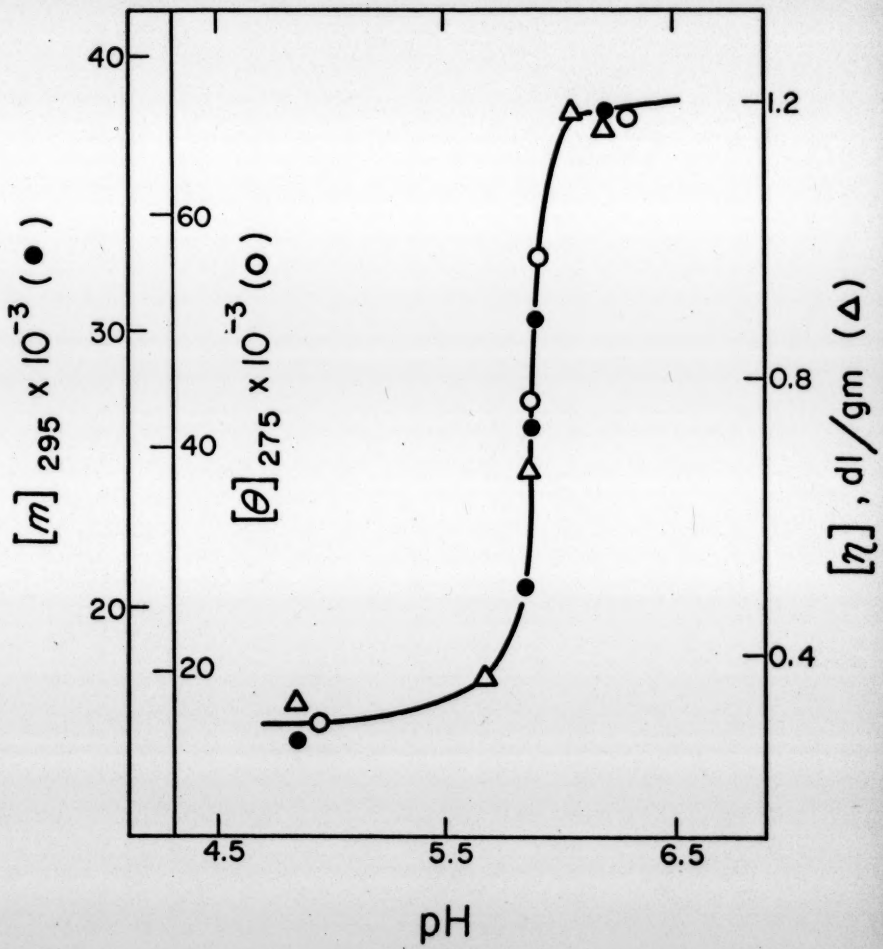
Hydrodynamic Properties of Poly(C)[a]

(in 0.08 M NaCl, 0.02 M Sodium Citrate plus Citric Acid at 25°C)

Solvent pH	[η] dl/gm	s° S	β x 10^{-6}
6.2	1.17	5.46	3.0
6.05	1.18	----	3.0
5.87	0.66	----	2.9
5.65	0.37	----	2.8
4.8	0.32	7.52	2.7
3.6	----	7.47	----

[a]Lot No. S: 6701

Figure 24: Variations of the intrinsic viscosities and CD and ORD extrema of poly(C) with pH in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid at 25°C. (Lot No. S: 6701)


40
30
20
60
40
20
1.2
0.8
0.4
4.5
5.5
6.5
pH
$[m]_{295} \times 10^{-3}$ (●)
$[\theta]_{275} \times 10^{-3}$ (○)
$[\eta]$, dl/gm (△)

symmetrical in acidic pH than at neutral pH.

3. Determination of Molecular Weight

By combining the intrinsic viscosity and the sedimentation coefficient measured at the same temperature, we estimated the molecular weights of poly(C) at pH 6.2 and 4.8 according to Equation (30) by using β-value of 3.0 and 2.7×10^6, respectively (as prolate ellipsoids of revolution). With $\bar{v} = 0.505$ (Gulik et al., 1970), the molecular weight turns out to be 9.6×10^4 at both pHs. Thus, it suggests that the acidic pH-induced conformational transition of poly(C) does not involve a change in the size of the polymer. Even if we choose a model of flexible coil and use Flory's $\Phi^{1/3} P^{-1}$ of 2.5×10^6, the estimated molecular weights will be 1.3 and 1.1×10^5 for pH 6.2 and 4.8, respectively. The agreement is not as good as that based on the β-function for an equivalent ellipsoid of revolution. Nevertheless, it still rules out the possibility of a dimerization of poly(C) in acidic solution.

D. Polyribouridylic Acid

1. Optical Studies

Figures 25 and 26 show the ORD and CD of poly(U) at pH 6.1 in 1.0 M KF, 0.1 M KF and solvent A (0.08 M NaCl and 0.02 M sodium citrate plus citric acid) at different temperatures. The ORD and CD are similar in these three salt concentrations at room temperature, but different at low temperatures. In 1.0 M KF, drastic changes in both magnitudes and positions of the extrema are observed between 24 and 1°C. Ts'o et al. (1966) also observed a change in ORD in 0.02 M $Mg(ClO_4)_2$ between 26 and 1°C and the magnitude was about 20% larger than ours at both temperatures. Similarly, Thrierr et al. (1971) reported a

Figure 25: ORD spectra of poly(U) at pH 6.1 and at different temperatures. The numerals refer to temperature in centigrade. ——— , 1.0 M KF;– – – , 0.1 M KF; •–•–• , 0.08 M NaCl and 0.02 M sodium citrate plus citric acid.

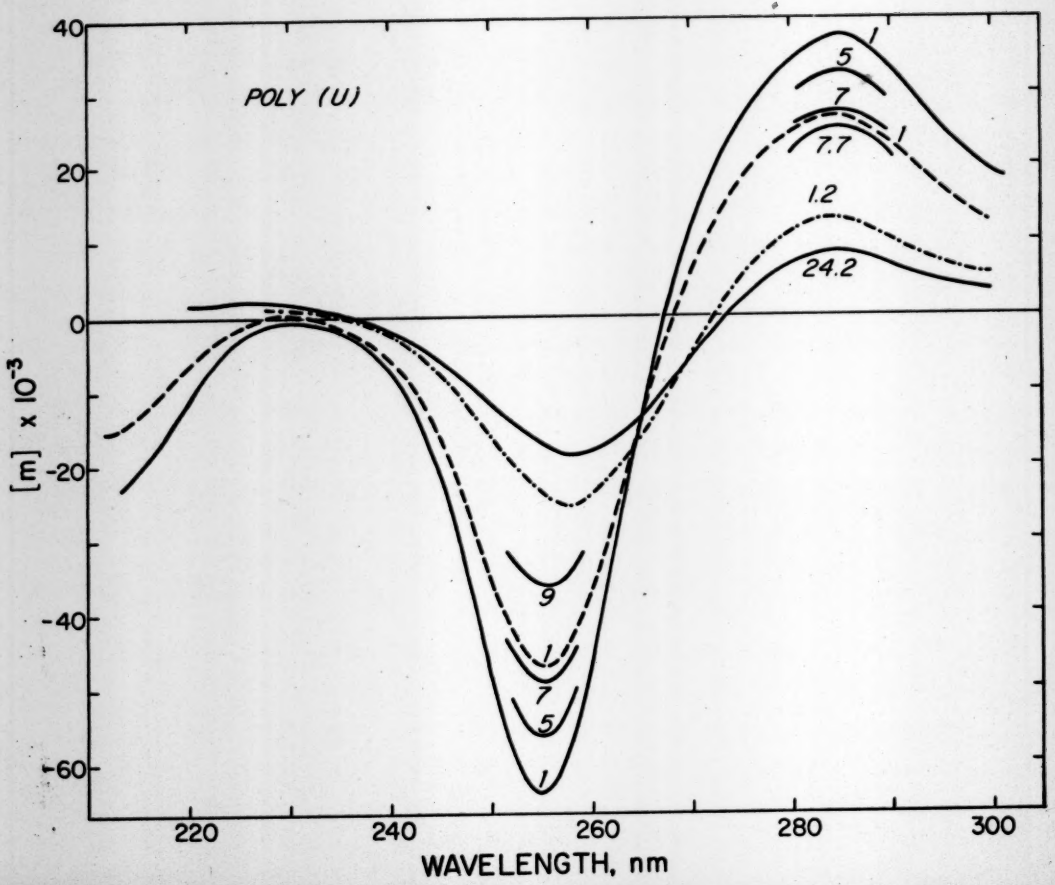
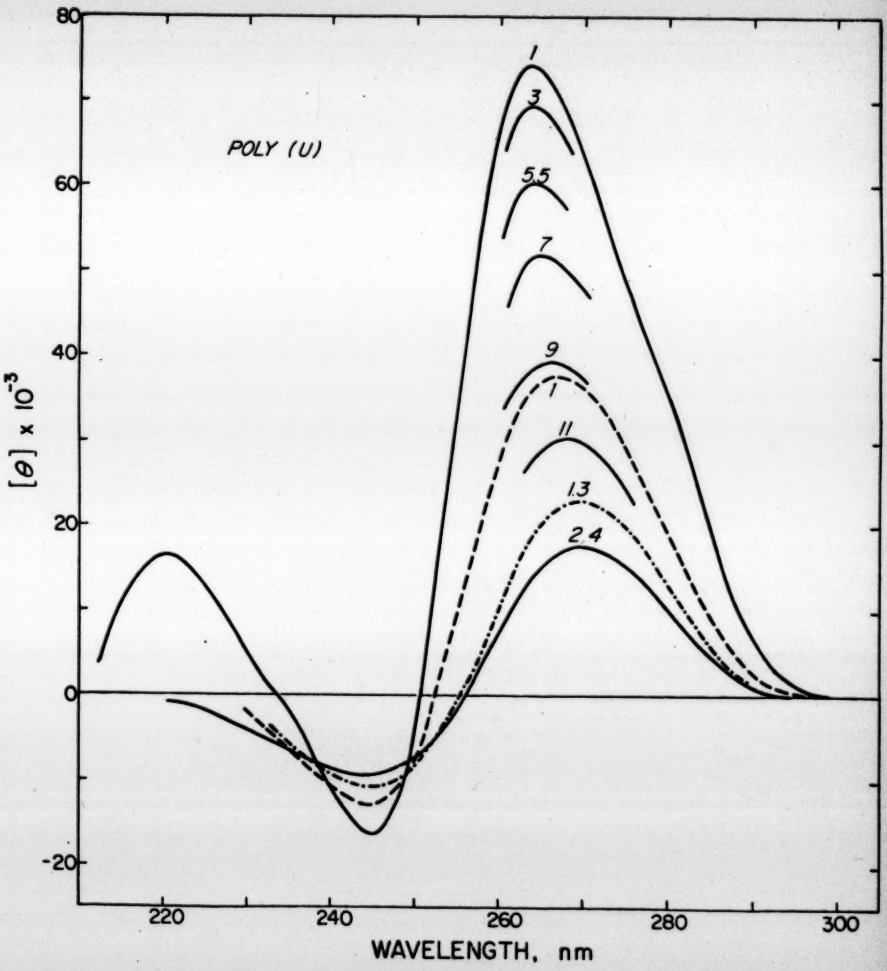

POLY (U)
40
20
0
-20
-40
-60
[m] x 10^-3
220
240
260
280
300
WAVELENGTH, nm
1
5
7
1
7.7
1.2
24.2
9
1
7
5
1

Figure 26: CD spectra of poly(U) at pH 6.1 and at different temperatures. Symbols, same as in Figure 25.


POLY (U)
[θ] x 10^-3
80
60
40
20
0
-20
220
240
260
280
300
WAVELENGTH, nm
1
3
5.5
7
9
11
13
2.4

transition in CD of comparable magnitude in 0.5 M CsCl between 35 and - 5°C. Both the trough of ORD and the positive band of CD on the long wavelength side show a significant blue-shift (5-10 nm) when temperature is lowered from 24 to 1°C. A similar temperature effect is observed for the ORD and CD in 0.1 M KF, but less drastically. In solvent A the Cotton effects show only slight increase in magnitude even at 1.2°C, suggesting little conformational changes, if any, upon lowering the temperature. Figure 27 shows the ORD and CD of poly(U) in 1.0 M KF below 230 nm at 25 and 2-3°C. At 25°C the ORD has two small positive shoulders at 227 and 204 nm. Ts'o et al. (1966) has reported a small positive band at 235 nm for poly(U) in 0.05 M $NaClO_4$ at 20°C. The CD has two small negative bands at 218 and 213 nm followed by a larger positive band at 199 nm. This is different from that in 0.1 M potassium phosphate reported by Wolfe et al. (1969), where poly(U) has a positive shoulder at 220 nm and a larger positive band near 195 nm. At 2-3°C the ORD of poly(U) has a large trough at 210 nm similar to that in 0.02 M Mg^{+2} (Ts'o et al., 1966) only our magnitude was 20% smaller, and the CD displays an intense positive band at 220 nm followed by a negative band at 203 nm.

The variations of the CD and ORD extrema of poly(U) in 1.0 M KF with temperature give a T_m = 8°C (Fig. 28). The melting temperature is much lower in 0.1 M KF. In solvent D (0.08 M KF and 0.02 M sodium citrate plus citric acid), the transition as followed by CD and absorbance changes is not complete even at zero degree.

2. Hydrodynamic Studies

Figure 29 shows the viscosity data of poly(U) measured at different temperatures. A viscometer with a flow time of 1,700 seconds for water

Figure 28: The temperature dependence of the ORD and CD extrema of poly(U) in 1.0 M KF at pH 6.1. Insert: the change of absorbance in 0.08 M KF and 0.02 M sodium citrate plus citric acid.

Figure 28: The temperature dependence of the ORD and CD extrema of poly(U) in 1.0 M KF at pH 6.1. Insert: the change of absorbance in 0.08 M KF and 0.02 M sodium citrate plus citric acid.

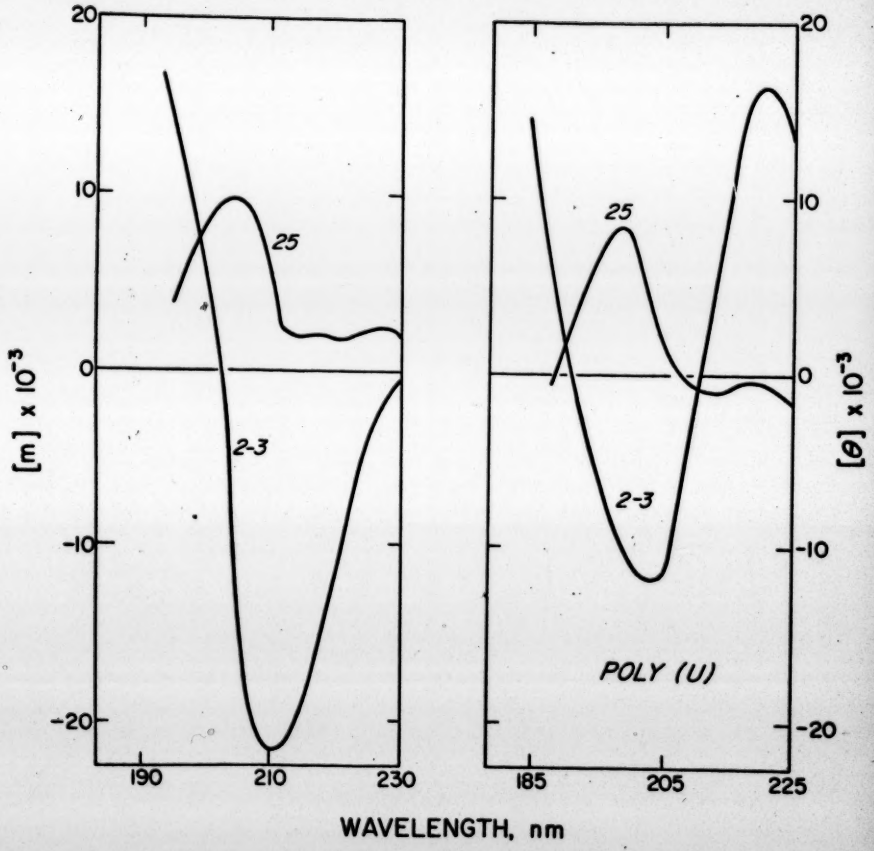

20
10
0
-10
-20
[m] x 10⁻³
25
2-3
190
210
230
185
205
225
[θ] x 10⁻³
POLY (U)
WAVELENGTH, nm

Figure 27: ORD and CD spectra of poly(U) in 1.0 M KF below 230 nm at 25°C and 2-3°C.

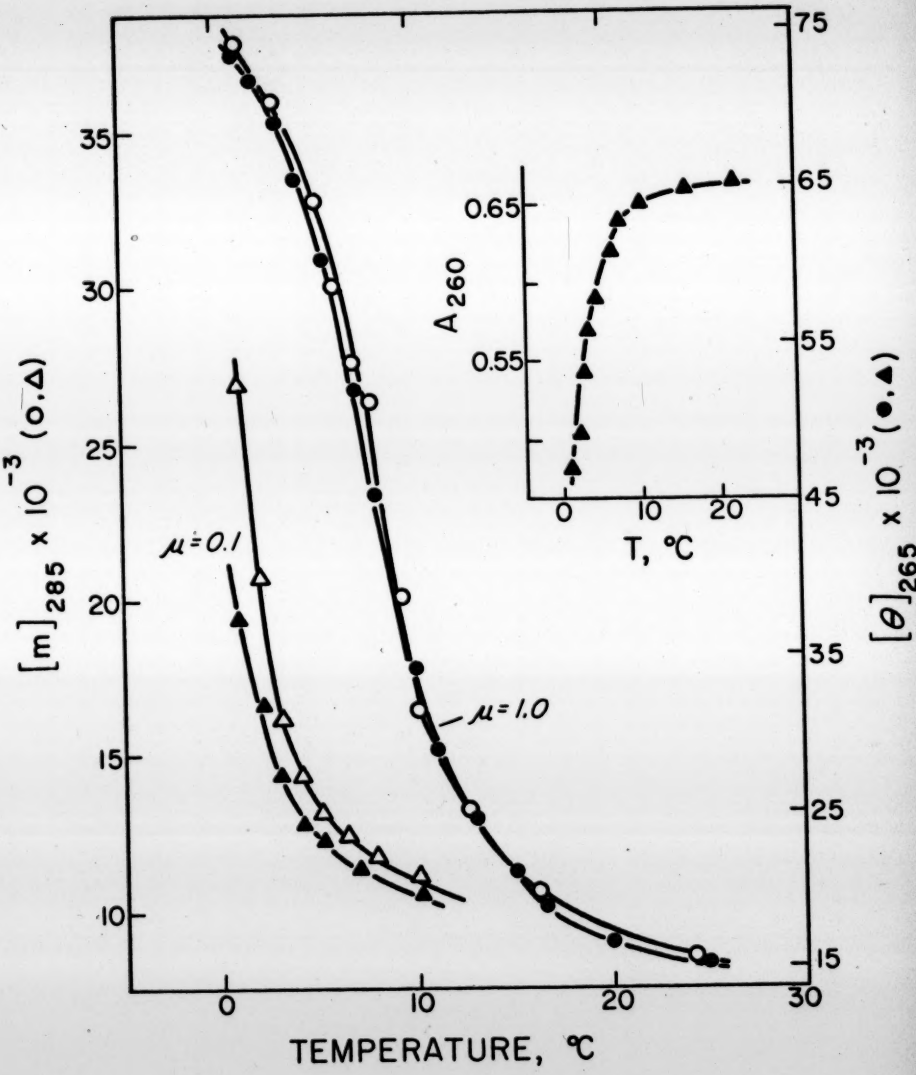
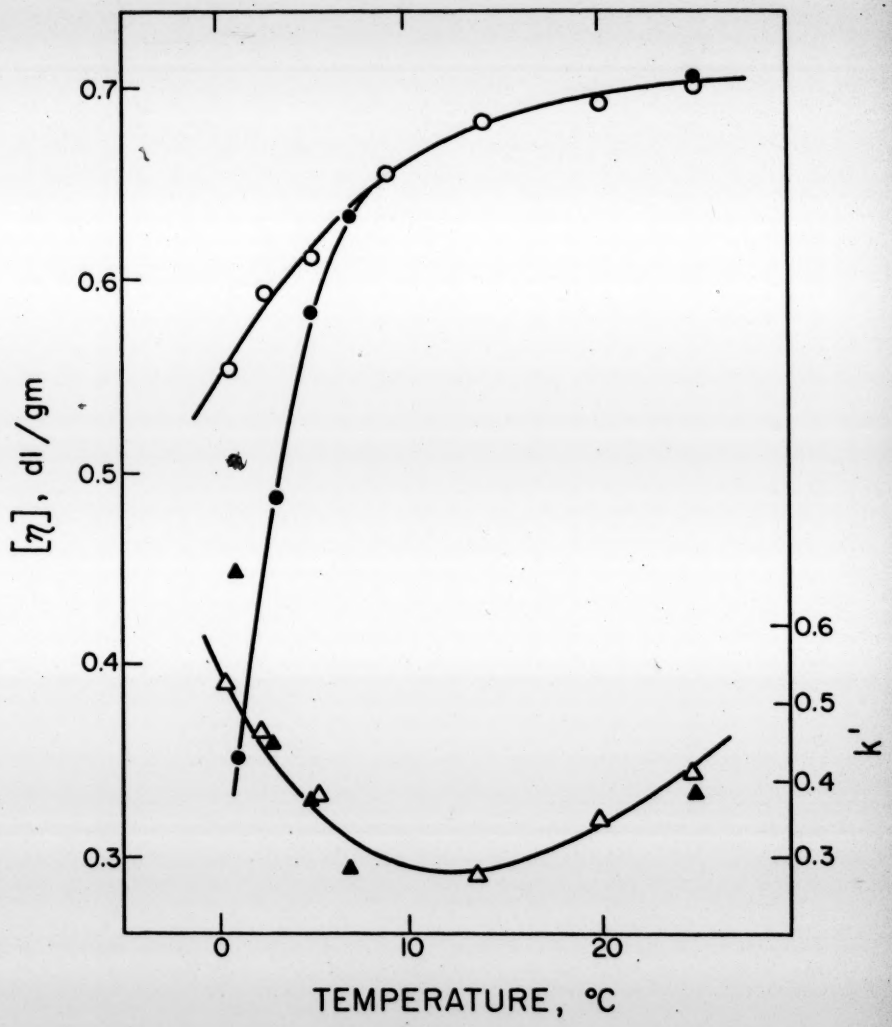

[m]285 x 10-3 (o.Δ)
[θ]265 x 10-3 (●.▲)
TEMPERATURE, °C
μ = 0.1
μ = 1.0
A260
T, °C
0.65
0.55
0
10
20
30
35
25
15
75
65
55
45

Figure 29: Intrinsic viscosity and Huggins' constants of poly(U) at different temperatures. ○ and △, in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid, pH 6.2; ● and ▲, same buffer except that KF replaced NaCl.


0.7
0.6
0.5
0.4
0.3
[η], dl/gm
0.6
0.5
0.4
0.3
k'
0
10
20
TEMPERATURE, °C

at 25°C was used and the concentration was so chosen that the difference in flow time between the solution and solvent was at least 100 seconds. The intrinsic viscosity decreases with the lowering of the temperature from 25 to 1°C in solvent A or D (pH 6.1). The Huggins' constant at all temperatures studied is less than one and shows a minimum value at about 10°C. KF appears to be more effective than NaCl in causing the intrinsic viscosity of poly(U) to decrease below 10°C.

The sedimentation coefficients of poly(U) were determined with both the schlieren optics and the photoelectric scanner at 3 and 25°C. In the former method, the s values were extrapolated to zero concentration; in the latter case, it was not necessary to correct the concentration dependence at such low concentration used (10^{-4} M). Both methods give virtually the same value of s°, which decreases with temperature; for instance, $s^{\circ}_{25} = 4.76$ S and $s^{\circ}_{3} = 3.01$ S in solvent D. When converted to standard conditions, the $s^{\circ}_{20,w}$ for poly(U) increases from 4.27 S at 25°C to 4.85 S at 3°C. The increase in $s^{\circ}_{20,w}$ as well as the drop in [η] suggests a more symmetrical structure at lower temperature. Solutions were always filtered through Millipore filters before use. In order to examine any effect on the s° by the different pore size of filters, poly(U) solution filtered through type HA (0.45 μ) filter, SM (5 μ) filter and without filtration was studied with the photoelectric scanner at 50,740 rpm and 25°C. The s° thus obtained is 4.9, 4.8 and 4.6 S, respectively. Thus, filtration through the Millipore filters does not affect the size distribution of the polymer. Table XI lists the hydrodynamic properties of poly(U) at different temperatures.

3. Determination of Molecular Weight

1. Sedimentation Equilibrium

Table XI

Hydrodynamic Properties of Poly(U)

(in 0.08 M KF, 0.02 M Sodium Citrate plus Citric Acid at pH 6.2)

Temperature °C	[η] dl/gm	s° S	β x 10^{-6}
25	0.7	4.76	2.9
18	---	4.37	---
7	0.63	3.82	2.9
5	0.57	2.76	2.8
3	0.50	3.01	2.8

Molecular weight of poly(U) in solvent D at pH 6.2 was determined at 20 and 8°C with the photoelectric scanner at 6,995 rpm. The data were analyzed by the "low-speed" method [Eq. (18)]. The weight-average molecular weight is the same at 20 and 8°C (Table XII).

ii. β-Function and $\Phi^{1/3} P^{-1}$ - Function

By combining the intrinsic viscosity and the sedimentation coefficient, the molecular weight was estimated according to Eq. (30). The partial specific volume, $\bar{v}$, was assumed unchanged with the temperature and a value of 0.564 (Inner & Felsenfeld, 1970) was used. The molecular weight determined by β- or $\Phi^{1/3} P^{-1}$-function is the same at 25 and 3°C, but the result based on β-function agrees better with that determined by sedimentation equilibrium (Table XII). Our results indicate that the changes in optical and hydrodynamic properties of poly(U) at low temperature do not involve a change in the molecular size, just as in the case of poly(C). Thrierr et al. (1971) suggest that poly(U) assumes a hairpin structure at low temperatures (see also Millar & Mackenzie, 1970).

Table XII

Molecular Weight Determination of Poly(U)

(in 0.08 M KF, 0.02 M Sodium Citrate plus Citric Acid at pH 6.2)

Method	Temperature °C	Molecular Weight x 10^{-4}
Sedimentation Equilibrium		
A (1) without filtration	20	8.5
(2) filtered through SM (5 μ) filter	20	8.0
B (1) without filtration	8	8.4
(2) filtered through HA (0.45 μ) filter	8	8.2
β-Function		
both	25	7.8
filtered through SM (5 μ) filter	3	8.3
$\phi^{1/3}p^{-1}$-Function		
both	25	9.5
filtered through SM (5 μ) filter	3	9.3

IV. DISCUSSION

A. Polyriboadenylic Acid

X-ray studies of poly(A) fibers drawn from concentrated acidic solution indicate that the structure is a double-stranded helix (Rich et al., 1961). It is therefore believed that the same structure is stable in acidic solution at room temperature. Our experimental measurements of the molecular weight of poly(A) in various pHs support the contention that the poly(A) molecule is dimerized upon protonation of the adenine bases. More extensive aggregation does occur, in particular, at very low pHs, but our measurements were confined to pH above 4.

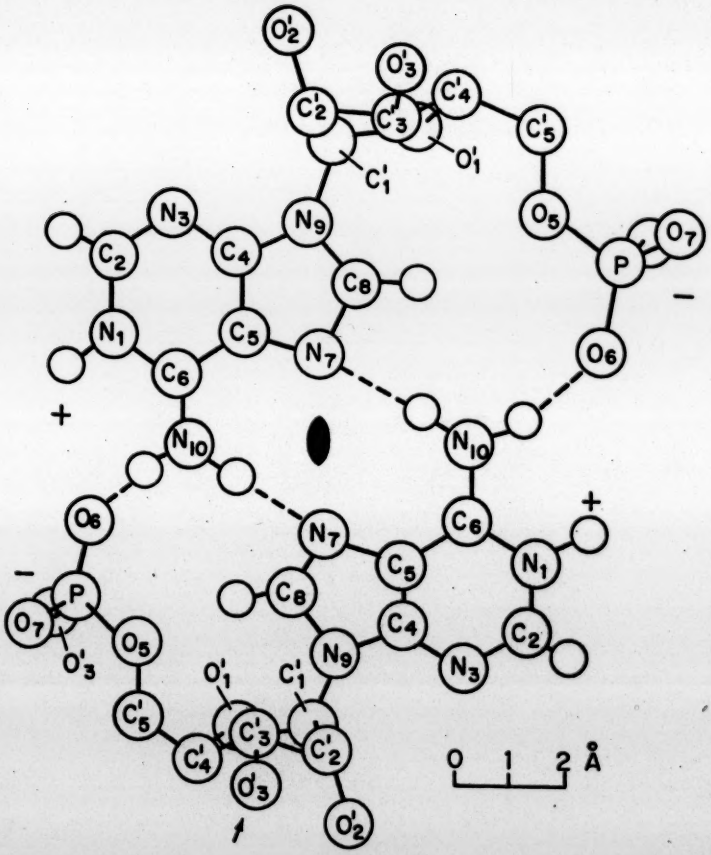
The ORD and CD of poly(A) have been extensively studied by many laboratories. The appearance of the multiple Cotton effects (Figs. 3-5) clearly indicates the presence of base stacking. The sharp temperature-induced transition of poly(A) in acidic solution is consistent with a cooperative melting process of double- to single-stranded form (Fresco & Klemperer, 1959; Holcomb & Tinoco, 1965; Sarkar & Yang, 1965a). Even the single-stranded poly(A) in neutral pH, whose melting curve is broad, is believed to form a flexible helix of stacked bases (Holcomb & Tinoco, 1965). It was Adler et al. (1969) who first detected two acidic forms, designated as A and B, on the basis of their ORD studies. However, the conformation of these two acidic forms were not specified. As can be seen from Fig. 3, the ORD peak on the long wavelength side is red-shifted together with an increase in magnitude when the poly(A) solution is acidified from pH 6.1 to 5.85 or 5.81. Lowering the pH of the solution to 4.2, however, reverses the trend to a blue shift but with still further increase in magnitude. More revealing is the ORD

curve at an intermediate pH, e.g., 5.4, which shows two positive peaks. Thus, this spectrum can not be explained with a mixture of only two forms, one for the single-stranded helix and the other double-stranded helix. Rather, it implies at least three forms, that is, one neutral and two acidic forms.

Our optical and hydrodynamic studies of poly(A) show a bell-shaped curve with respect to pH (Fig. 6). The midpoint of the transition on the long wavelength side is located at pH 5.7-5.9 in 0.08 M NaCl and 0.02 M sodium citrate plus citric acid. From potentiometric titration study Adler et al. (1969) reported a pk_a of 5.8-5.9 for the adenine bases in poly(A) in 0.1 M NaCl. Thus, the acidic B form is probably a half-protonated double-stranded helix. The X-ray studies of poly(A) fibers (Rich et al. 1961) suggest that the double-stranded helix is stabilized through four hydrogen bonds plus two salt linkages (see Fig. 30). The potentiometric titration study of poly(A) also shows that the adenine bases are fully protonated near pH 4, where the acidic A form occurs. Our results, however, suggest that partial protonation is sufficient to stabilize the double-stranded helical B form. Full protonation of the adenine bases would lead to a different double-stranded helical A form.

Since poly(A) has one negative charge per phosphate residue at neutral pH, the single-stranded polymer can be considered as a flexible polyelectrolyte. When the adenine bases are partially protonated, the positive charge on the N(1) would interact with the negative charge on the phosphate group; therefore the electrostatic repulsion among the phosphate groups is, in part, decreased. This decrease in the electrostatic repulsion might promote the interactions between the strands. Thus, the transition from the single-stranded poly(A) to the half-protonated

Figure 30: The double-stranded molecule of poly(A) as viewed down the fiber axis (Rich *et al.*, 1961).


O'2
O'3
C'4
C'2
C'3
C'5
O'1
C'1
N3
N9
O5
O7
C2
C4
C8
P
N1
C5
N7
O6
C6
N10
N10
O6
N7
C6
C5
N1
P
C8
C4
C2
O7
O5
N9
N3
O'3
O'1
C'1
C'5
C'3
C'2
C'4
O'3
O'2
0 1 2 Å

double-stranded acidic B form would render the B form a more extended (i.e. less flexible) helix. This could account for the observed sharp increase in the intrinsic viscosity when the pH of the solution is lowered from neutral to 5.81 (Fig. 6). When the adenine bases are fully protonated, the double-stranded helical A form occurs and the net charge on the poly(A) molecule becomes zero (one positive charge on the base and one negative on the phosphate per nucleotide). The salt linkage between $N(1)^{+}H$ and PO_4^{-} of the opposite strands might facilitate the H-bonding between O(6) and N(10)H, so that the acidic A form is stabilized by two salt linkages and four hydrogen bonds (Fig. 30). Thus, this A form would be more compact and less asymmetric than the B form. It is therefore not surprising to find that the intrinsic viscosity drops again below pH 5. On the other hand, the overall multiple Cotton effects (Figs. 3-5) remain strong in all acidic pHs and, furthermore, both the acidic A and B forms have the same hypochromicity relative to the single-stranded form in neutral pH. These findings suggest the base stacking of the poly(A) molecule remains undisrupted even after full protonation. This is also clear from the model shown in Fig. 30, where the two positive charges are far apart from each other and will not destabilize the double-stranded helix through electrostatic repulsion.

Our conclusion differs slightly from that of Adler *et al.* (1969), who proposed that the B form was the only structure at pH 5.81 in 0.1 M NaCl, and that a mixture of A and B forms existed between pH 5.8 and 4.0. We believe that the B form is stable between pH 5.2 and 5.7 under our experimental conditions. Above pH 5.7, the double-stranded poly(A) undergoes a cooperative transition to a single-stranded form; below pH 5.2, there exists another cooperative transition to a

different double-stranded helix. Holcomb and Tinoco (1965) have studied the temperature effect on the specific viscosity of poly(A) in both neutral and acidic pHs. Replotting their η_{sp} at a concentration of 0.024% at four pHs also led to a bell-shaped curve, such as that shown in Fig. 6. However, direct comparison with our results is not possible because of the lack of intrinsic viscosity data in their work. Neither could we explain their finding that the η_{sp} approached zero at pH 4.6, which these authors attributed to extensive aggregation.

Sedimentation equilibrium with Rayleigh optics is by far one of the most precise methods to determine molecular weight. However, sedimentation equilibrium with photoelectric scanner eliminates the correction for concentration dependence because of the use of very dilute polymer solution, and was thus utilized. Since our interest was not in the absolute molecular weight of the polymer, but rather in the change in molecular size relative to conformational changes of poly(A) in acidic solution, the scanner system was thought to be adequate for our purpose. In addition, we also estimated the molecular weight from hydrodynamic measurements. The results in Table III clearly show that the two methods give comparable results.

The disadvantage of viscometry is that polymer concentration must be moderately high to obtain precise measurements. Nevertheless, it is one of the simplest tools for studying the shape of polymers in solution. In connection with the sedimentation coefficient, it can also give a reasonable estimate of the molecular weight through the use of Equation (28) or (30). Eisenberg and Felsenfeld (1967) studied the molecular weight (by light scattering), $[\eta]$ and s° of poly(A) at various temperatures and concluded that the β-value [Equation (30)] changed with temperature. Thus, they questioned the common procedure

of using an assumed fixed β-value in the determination of molecular weight. Actually, the selection of a constant β-value in such calculations is justified only for a flexible coil model using Flory's $\Phi^{1/3}P^{-1}$ (which is identical in form with the β-function). Our results, however, have shown that an equivalent prolate ellipsoid is a better model to represent the poly(A) molecule. Clearly, the β-value would then vary with the assumed axial ratio of the ellipsoid. Eisenberg and Felsenfeld (1967) found that the intrinsic viscosity of poly(A) decreased and its sedimentation coefficient increased with increasing temperature. This would lead to a gradual diminution of the β-value at high temperature, as it should be. Indeed, there is no justification to assume a fixed β-value in this case. Our results in Table III fully support our conclusion.

B. Polyriboguanylic Acid

Fresco and Massoulie' (1963) first reported that poly(G) at neutral pH tended to aggregate and form a multiple-stranded helix with an extremely high thermal stability. Pochon and Michelson (1965), pointed out the lack of any evidence that supported the presence of more than two strands for the poly(G) complex. Furthermore, Michelson et al. (1967) found that the solution of an oligoguanylic acid at a concentration of 7 mg/ml was extremely viscous and they suspected that the preparation of Fresco and Massoulie' (1963) might be of relatively short chain length which behaved differently from poly(G). However, our viscosity measurements of a poly(G) preparation having a molecular weight of about 2×10^5 (Table XIII) showed a moderate intrinsic viscosity of 0.33 dl/gm at 25°C (Table VII). Nevertheless, our molecular weight determination of poly(G) indicates that it is aggregated and

consists of multiple strands at neutral pH (Table VIII). Both the intrinsic viscosity and sedimentation coefficient dropped significantly upon raising the pH of the solution from 7 to 11.3 (Table VII). The corresponding molecular weight, as estimated from the β-function or the $\Phi^{1/3}P^{-1}$-function, was only 1/5 or 1/6 of that at neutral pH. The s° value dropped even further at pH 11.6, suggesting a further reduction in molecular size. Thus, evidence strongly suggests that poly(G) at neutral pH is in a highly aggregated form.

Among the polyribonucleotides studied, only poly(G) in alkaline solution (pH > 11) was found to have little, if any, base stacking in the polymer chain, as revealed by the loss of multiple Cotton effects (see Figs. 10 and 11). The optical studies of poly(G) showed a sharp transition above pH 11 (Figs. 9 and 13). This is likely to be due to the partial dissociation of the enol form of guanine [-N(1)=C(6)-OH], which would increase the negative charges on the nucleotide residues. If the base is fully deprotonated, each nucleotide residue would carry two negative charges. As a result, the electrostatic repulsion would be so strong that the bases would be forced to unstack. Furthermore, dis-aggregation of the polymer also occurred in alkaline solution as a time-dependent reaction illustrated by the gel chromatography (Fig. 14). This is consistent with the corresponding time-dependent changes in the ORD and CD spectra (see Table IV).

Poly(G) in acidic solution displays multiple Cotton effects (Figs. 16 and 17) as well as little change in its absorption spectrum (Fig. 8). Notable changes in the CD spectra at low pHs are the gradual disappearance and, finally, inversion of the positive shoulder near 290 nm. (The negative CD band at 240 nm also turns into a

positive minimum). Similarly, the ORD shoulder near 300 nm turns into a negative trough, but the magnitude of the 270-nm peak increases with the lowering in pH. Plots of these extrema versus pH (Fig. 18) shows a sharp transition with a midpoint near pH 3. These changes could be attributed to the protonation of guanine at position N(7). The acquisition of a positive charge in addition to the negative charge on the phosphate group would result in a reduction of the electrostatic repulsion among the nucleotide residues. Since the number of strands in poly(G) is uncertain, and the structure of the aggregates unknown, it is difficult to speculate on the kind of conformational changes which have taken place as a result of acidification.

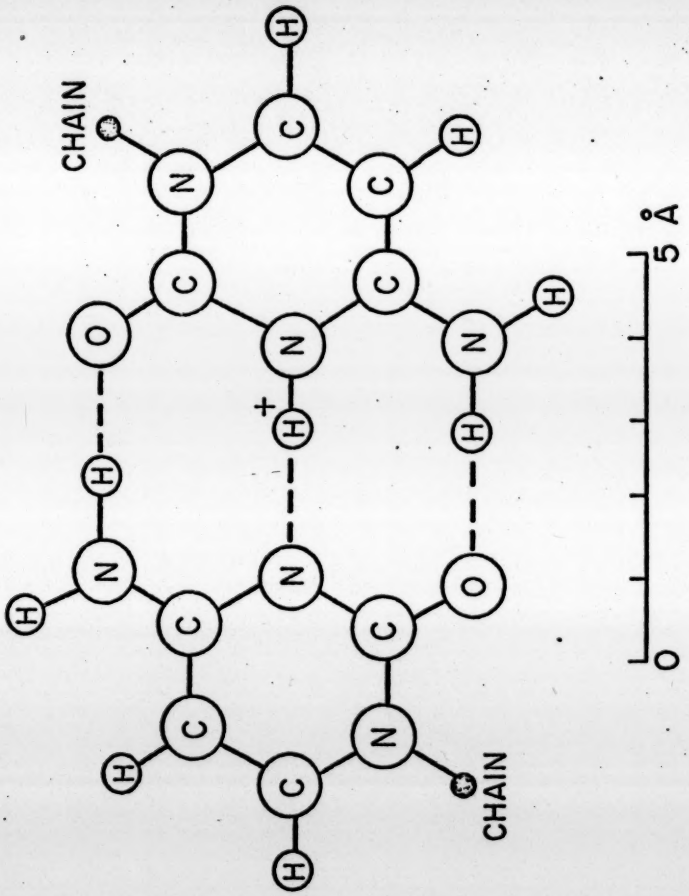
Our hydrodynamic studies indicate that the viscosity of poly(G) is only slightly reduced, and the sedimentation coefficient modestly increased in the acidic transition region (Fig. 18). For instance, the specific viscosity for a 0.04% solution dropped from 0.34 dl/gm at pH 7 to 0.30 dl/gm at pH 3.1. Because of the exhaustion of the poly(G) sample, we were unable to determine its intrinsic viscosity. However, it is expected to be somewhat lower than that at neutral pH. On the other hand, the s°_{25} increased from 8.1 S at pH 7 to 9.0 S at pH 2.8. These results rule out any drastic change in the overall shape of poly(G) in acidic solution. Furthermore, according to the β-function in Equation (30), the findings also suggest little change in the molecular weight of poly(G) in acidic transition region. Unlike alkaline solution, the optical changes of poly(G) in acidic solution are reversible and time-independent. These findings again demonstrate the marked difference in the conformation and molecular shape of poly(G) in acidic and alkaline solutions.

Finally, the absorption spectra of poly(G) in Fig. 8 shows a red shift of the shoulder near 280 nm in acidic solution. If this shoulder is taken as an n-π^* electronic transition, as suggested for poly(A) by Bush and Scheraga (1969), this red shift would imply that in acidic solution the bases of poly(G) are buried in a less polar environment than that at neutral pH, that is, they are more shielded from the polar solvent. This is consistent with the contention that the poly(G) molecules contract to some extent in acidic solution. On the other hand, in alkaline solution the 280-nm shoulder disappears and is concealed in the major absorption band (Fig. 8). If anything, it seems to be slightly blue-shifted. This would imply that the bases are exposed to a more polar environment in alkaline medium, a contention in accord with the dissociation of the poly(G) aggregates in alkaline solution.

C. Polyribocytidylic Acid

The ORD and CD spectra of poly(C) have also been reported by many laboratories. It is generally believed that the polynucleotide molecule is single-stranded in neutral solution, and is converted into a double-stranded complex in acidic solution. According to the X-ray study of fibers drawn from a concentrated acidic solution (Langridge and Rich, 1963), poly(C) forms a double-stranded helix with two hydrogen bonds between $-C(4)NH_2$ and $-C(2)O$ groups per each base pair. The helix is further stabilized by a positive charge on N(3) (Fig. 31). From a potentiometric titration study, Hartman and Rich (1965) reported a pK_a value of 5.7 for the cytosine bases in the polymer (Miles Laboratories, Inc.). Our optical studies of poly(C) from Miles Laboratories, Inc. also show a cooperative transition in acidic solution

Figure 31: Hydrogen bonding between the cytosine bases in poly(C) (Langridge & Rich, 1963).


CHAIN
CHAIN
0
5 Å

with a midpoint at pH 5.7 (not shown), and both optical and hydrodynamic data of sample from Schwarz BioResearch, Inc. show that at pH 5.8 (Fig. 24), suggesting that the acidic form is very likely to be a half-protonated double-stranded helix (see Discussion below). The transition is so sharp that the ORD spectra in the transition region can easily be shifted; for instance, the twin peaks at pH 5.87 tend to change toward the spectrum at neutral pH merely by shaking the poly(C) solution. Nevertheless, there is no evidence for the collapse of the poly(C) structure, such as the disappearance of the rotations that had been noted by Guschlbauer (1967) for solutions at a pH slightly above the pK_a value.

Figure 21 seems to show an isorotational point near 300 nm, a fact indicative of the presence of two forms, one at neutral pH and the other in acidic solution. Unlike poly(A) (Section IV A), poly(C) can not have two acidic forms (one half-protonated and the other fully protonated). The explanation may be found from the diagram in Fig. 31, where a double protonation on N(3) would put two positive charges adjacent to each other and the resultant electrostatic repulsion would disrupt the double-stranded helix.

The important finding in this work is that the molecular weight of poly(C) does not change with change in conformation (see III C3). (We again used the β-function for estimating the molecular weight of poly(C).) A possible explanation is that poly(C) forms a hairpin structure upon protonation, rather than a double-stranded dimer as in the case of poly(A). This conclusion differs from the X-ray results of poly(C) fibers which inferred a parallel double-stranded dimer. However, the fibers were drawn from a concentrated polymer solution, in which poly(C) could conceivably have a conformation that is

different from that in a very dilute solution. Furthermore, two hairpin-like molecules could be stretched during drawing into a parallel double-stranded complex.

In neutral solution poly(C) has one negative charge per phosphate group. Thus, the molecule is expected to be rather extended, albeit flexible. Upon half protonation the molecules would tend to contract if they adopt a hairpin model. This would also account for the marked drop in the intrinsic viscosity and a gradual increase in the sedimentation coefficient in the transition region. This is unlike the extended double-stranded helix of poly(A) (the acidic B form) which displays an unusually high intrinsic viscosity.

In general, the formation of base pairs in RNA leads to a blue shift of its ORD and CD spectra (Yang & Samejima, 1969). Exceptions may be found in cases where the polynucleotides are protonated or deprotonated with resultant changes in their absorption spectra. For example, CMP shows a red shift in its absorption spectrum when the base is protonated. Thus, the ORD and CD spectra of poly(C) in acidic solution is also red-shifted relative to those in neutral solution, even though the cytosine bases of the polynucleotide are paired in the acidic form.

D. Polyribouridylic Acid

Poly(U) is known to have little secondary structure at room temperature, even though some stacking of the uracil bases does exist as revealed by the appearance of multiple Cotton effects in the ORD and CD spectra (Figs. 25 and 26). Lowering the temperature of the solution (toward 0°C) causes a thermal transition of the poly(U) molecule from a less ordered to a more ordered structure.

The blue shift of the positive CD band on the long wavelength side (Fig. 26) is indicative of the formation of base pairs (Yang & Samejima, 1969), probably through the two hydrogen bonds between -c(4)O or -C(2)O and -N(3)H of the opposite bases (Fig. 1).

This temperature-induced transition of poly(U) is dependent on the nature of salts present as well as the concentration of salts used. There is some controversy concerning a salt-dependent aggregation (Brown, 1966; Millar & Mackenzie, 1970). According to Millar and Mackenzie (1970), aggregation occurred above 0.3 M KCl, which would complicate the interpretation of the size of poly(U) under various experimental conditions. For this reason, we studied the optical and hydrodynamic properties of poly(U) in 0.08 M KF and 0.02 M sodium citrate plus citric acid. The disadvantage of using low salts is the incomplete thermal transition of poly(U), even at 0°C (Figs. 28 and 29). Nevertheless, the data are sufficient to detect the change, if any, in molecular size of poly(U) in the transition region. From both sedimentation equilibrium, and the β-function analyses, (Table XII), it was found that the molecular weight of poly(U) remained unchanged over the temperature range studied (0-25°C). In addition, the intrinsic viscosity of poly(U) dropped markedly with decreasing temperature, suggesting that the polymer is less asymmetric at lower temperature. Thus, our results support the current model of poly(U), i.e., a hairpin structure at low temperature (near 0°C) with the polynucleotide chain folded back upon itself, but a single-stranded chain with little secondary structure at higher temperature, e.g., room temperature.

V. SUMMARY

The molecular weights and hydrodynamic properties of four polyribonucleotides indicate that the conformational changes of these polymers under various conditions are accompanied by changes in size and shape.

Poly(A) is dimerized upon protonation of the adenine bases. There are two double-stranded forms of poly(A), one a half-protonated B form and the other a fully protonated A form. The acidic A form is more compact and more symmetric than the B form as suggested by the lower intrinsic viscosity of the A form.

Poly(G) at neutral pH is highly aggregated and shows an extremely high thermal stability. In alkaline solutions above the pK_a value, it undergoes a time-dependent disaggregation and shows little, if any, base stacking in the polymer chain. Upon protonation poly(G) shows some changes in conformation, but no evidence of disaggregation.

Upon half protonation poly(C) appears to form a hairpin structure. Supporting data are the marked drop in its intrinsic viscosity, the gradual increase in its sedimentation coefficient, and the lack of change in molecular weight.

The fourth polymer, poly(U), shows some degree of base stacking at room temperature. Our results support the current hypothesis that it assumes a hairpin structure at low temperature.

VI. REFERENCES

Adler, A., Grossman, L., and Fasman, G. D. (1967). Proc. Nat. Acad. Sci. U. S., 57, 423.

Adler, A., Grossman, L., and Fasman, G. D. (1968). Biochemistry, 7, 3836.

Adler, A. J., Grossman, L., and Fasman, G. D. (1969). Biochemistry, 8, 3846.

Akinrimisi, C., Sander, C., and Ts'o, P. O. P. (1963). Biochemistry, 2, 340.

Allen, R. J. L. (1940). Biochem. J., 34, 858.

Arya, S. K. (1968). Ph.D. Dissertation, University of California, San Francisco.

Beers, R. F., Jr., and Steiner, R. F. (1957). Nature, 179, 1076.

Bradbury, J. H. (1970). In "Physical Principles and Techniques of Protein Chemistry," (Leach, S. J., ed.), Academic Press, New York, Part B. p. 99.

Brahms, J. (1963). J. Amer. Chem. Soc., 85, 3298.

Brahms, J. (1964). Nature, 202, 797.

Brahms, J., and Brahms, S. (1970). In "Fine Structure of Proteins and Nucleic Acids," (Fasman, G. D., and Timasheff, S. N., eds.), Marcel Dekker, New York, p. 191.

Brahms, J., Maurizot, J. C., and Michelson, A. M. (1967). J. Mol. Biol., 25, 465.

Brahms, J., Michelson, A. M., and Van Holde, K. E. (1966). J. Mol. Biol., 15, 467.

Brahms, J., and Mommaerts, W. F. H. M. (1964). J. Mol. Biol., 10, 73.

Brahms, J., Sellini, H., and Brahms, S. (1969). III Intern. Biophys. Congr. Abstr., p. 171.

Brown, R. A. (1966). Arch. Biochem. Biophys., 115, 102.

Bush, C. A., and Scheraga, H. A. (1969). Biopolymers, 7, 395.

Cantor, C. R., Jaskunas, S. R., and Tinco, I., Jr. (1966). J. Mol. Biol., 20, 39.

Cantor, C. R., and Tinco, I., Jr. (1965). J. Mol. Biol., 13, 65.

Cassim, J. Y., and Yang, J. T. (1969). Biochemistry, 8, 1947.

Chen, P. S., Toribara, T. Y., and Warner, H. (1956). Anal. Biochem., 28, 1756.

Cohen, P., Chin, R. C., and Kidson, C. (1969). Biochemistry, 8, 3603.

Davidson, B., and Fasman, G. D. (1971). Arch. Biochem. Biophys., 144, 650.

Dekker, C. A. (1960). Ann. Rev. Biochem., 29, 453.

Eisenberg, H., and Felsenfeld, G. (1967). J. Mol. Biol., 30, 17.

Fasman, G. D., Lindblow, C., and Grossman, L. (1964). Biochemistry, 3, 1015.

Felsenfeld, G., and Miles, H. T. (1967). Ann. Rev. Biochem., 36, 407.

Fresco, J. R. (1959). J. Mol. Biol., 1, 106.

Fresco, J. R., and Doty, P. (1957). J. Amer. Chem. Soc., 79, 3928.

Fresco, J. R., and Klemperer, E. (1959). Ann. N. Y. Acad. Sci., 81, 730.

Fresco, J. R. and Massoulie', J. (1963). J. Amer. Chem. Soc., 85, 1352.

Green, G., and Mahler, H. R. (1970). Biochemistry, 9, 368.

Gulik, A., Inoue, H., and Luzzati, V. (1970). J. Mol. Biol., 53, 221.

Guschlbauer, W. (1967). Proc. Nat. Acad. Sci. U.S., 57, 1441.

Hartman, K. A., Jr., and Rich, A. (1965). J. Amer. Chem. Soc., 87, 2033.

Hashizume, H., and Imahori, K. (1967). J. Biochem. (Tokyo), 61, 738.

Helmkamp, G. K., and Ts'o, P. O. P. (1962). Biochim. Biophys. Acta, 55, 601.

Holcomb, D. N., and Timasheff, S. N. (1968). Biopolymers, 6, 513.

Holcomb, D. N., and Tinoco, I., Jr. (1965). Biopolymers, 3, 121.

Huggins, M. L. (1942). J. Amer. Chem. Soc., 64, 2716.

Inman, R. B., and Baldwin, R. L. (1962). J. Mol. Biol., 5, 172.

Inners, L. D., and Felsenfeld, G. (1970). J. Mol. Biol., 50, 373.

Lamborg, M. R., Zamecnik, P. C., Li, T. K., Kagi, J., and Vallee, B. L. (1965). Biochemistry, 4, 63.

Langridge, R., and Rich, A. (1963). Nature, 198, 725.

Leng, M., and Felsenfeld, G. (1966). J. Mol. Biol., 15, 455.

Leng, M., and Michelson, A. M. (1968). Biochim. Biophys. Acta, 155, 91.

Lipsett, M. N. (1960). Proc. Nat. Acad. Sci. U.S., 46, 445.

Lowry, T. M. (1935). In "Optical Rotatory Power," Green, London; Dover Publications (1964).

Luzzati, V., Mathis, A., Masson, F., and Witz, J. (1964). J. Mol. Biol., 10, 28.

Mandelkern, L., and Flory, P. J. (1952). J. Chem. Phys., 20, 212.

Mandelkern, L., Krigbaum, W. R., Scheraga, H. A., and Flory, P. J. (1952). J. Chem. Phys., 20, 1392.

Michelson, A. M. (1963). In "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, p. 456 and 508.

Michelson, A. M., Massoulie', J., and Guschlbauer, W. (1967). Progr. Nucl. Acid Res. Mol. Biol., 6, 84.

Michelson, A. M., and Monny, C. (1966). Proc. Nat. Acad. Sci. U.S., 56, 1528.

Michelson, A. M., Ulbricht, T. L. V., Emerson, T. R., and Swan, R. J. (1966). Nature, 209, 873.

Millar, D. B., and Mackenzie, M. (1970). Biochim. Biophys. Acta, 204, 82.

Oncley, J. L. (1941). Ann. N. Y. Acad. Sci., 41, 121.

Perrin, F. (1936). J. Phys. Radium, 7, 1.

Pochon, F., and Michelson, A. M. (1965). Proc. Nat. Acad. Sci. U.S., 53, 1425.

Rawitscher, M. A., Ross, P. D., and Sturtevant, J. M. (1963). J. Amer. Chem. Soc., 85, 1915.

Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D. (1961). J. Mol. Biol., 3, 71.

Richards, E. G., Flessel, C. P., and Fresco, J. R. (1963). Biopolymers, 1, 431.

Saito, H., and Muira, K. (1963). Biochim. Biophys. Acta, 72, 619.

Sarkar, P. K., and Yang, J. T. (1965a). J. Biol. Chem., 240, 2088.

Sarkar, P. K., and Yang, J. T. (1965b). Biochemistry, 4, 1238.

Sarocchi, M-T., Courtois, Y., and Guschlbauer, W. (1970). Eur. J. Biochem., 14, 411.

Schachman, H. K. (1957). Methods in Enzymology, vol. IV, p. 32.

Schachman, H. K. (1959). "Ultracentrifugation in Biochemistry," Academic Press, New York.

Scheraga, H. A., and Mandelkern, L. (1953). J. Amer. Chem. Soc., 75, 179.

Schneider, W. C. (1957). Methods in Enzymology, vol. III, p. 680.

Shapiro, R. (1968). Progr. Nucl. Acid Res. Mol. Biol., 8, 73.

Simha, R. (1940). J. Phys. Chem., 44, 25.

Simpkins, H., and Richards, E. G. (1967a). J. Mol. Biol., 29, 349.

Simpkins, H., and Richards, E. G. (1967b). Biopolymers, 5, 551.

Steiner, R. F., and Beers, R. F., Jr. (1957). Biochim. Biophys. Acta, 26, 336.

Steiner, R. F., and Beers, R. F., Jr. (1959). Biochim. Biophys. Acta, 32, 166.

Steiner, R. F., and Beers, R. F., Jr. (1961). In "Polynucleotides," Elsevier, Amsterdam, chapt. 8.

Svedberg, T., and Pedersen, K. O. (1940). In "The Ultracentrifuge," Oxford, Clarendon Press.

Tennent, H. G., and Vilbrandt, C. F. (1943). J. Amer. Chem. Soc., 65, 424.

Thiele, D., and Guschlbauer, W. (1971). Biopolymers, 10, 143.

Thrierr, J. C., and Dourlent, M., and Leng, M. (1971). J. Mol. Biol., 58, 815.

Thrierr, J. C., and Leng, M. (1969). Biochim. Biophys. Acta, 182, 575.

Tinoco, I., Jr. (1964). J. Amer. Chem. Soc., 86, 297.

Tinoco, I., Jr., and Cantor, C. R. (1970). Methods of Biochemical Analysis, 18, 81.

Ts'o, P. O. P., Helmkamp, G. K., and Sander, C. (1962). Biochim. Biophys. Acta, 55, 584.

Ts'o, P. O. P., Rapaport, S. A., and Bollum, F. J. (1966). Biochemistry, 5, 4153.

Tsuboi, M., Kyogoku, Y., and Shimanonchi, T. (1962). Biochim. Biophys. Acta, 55, 1.

Ulbricht, T. L. V., Swan, R. J., and Michelson, A. M. (1966). Chem. Commun., 63.

Van Holde, K. E. (1967). "Sedimentation Equilibrium," Fractions No. 1, Published by Spinco Division of Beckman Instruments, Inc.

Van Holde, K. E., Brahms, J., and Michelson, A. M. (1965). J. Mol. Biol., 12, 726.

Warner, R. C. (1957). J. Biol. Chem., 229, 711.

Warshaw, M. M., Bush, C. A., and Tinoco, I., Jr. (1965). Biochem. Biophys. Res. Commun., 18, 633.

Williams, J. W., Van Holde, K. E., Baldwin, R. L., and Fujita, H. (1958). Chem. Rev., 58, 715.

Witz, J., and Luzzati, V. (1965). J. Mol. Biol., 11, 620.

Wolfe, F. H., Oikawa, K., and Kay, C. M. (1969). Can. J. Biochem., 47, 637.

Yang, J. T. (1961). Advan. Protein Chem., 16, 323.

Yang, J. T. (1968). In "A Laboratory Manual of Analytical Methods in Protein Chemistry," (Alexander, P., and Lundgren, H. P., eds.), Pergamon Press, Oxford, vol. 5, chapt. 2.

Yang, J. T., and Samejima, T. (1969). Progr. Nucl. Acid Res. Mol. Biol., 9, 223.

Yphantis, D. A. (1964). Biochemistry, 3, 297.